Pak J Sci Ind Res 1998 41 (2) 63-67

POTENTIAL ANTIBACTERIAL AGENTS *PART-IV.* SYNTHESIS OF NEW 1[2-PYRIMIDINO]-AMINO-METHYL BENZIMIDAZOLES AND 1,2, 3 BENZOTRIAZOLES

Shaheen A Hussain*[a], Tahira B Sarfaraz[a], Najma Murtaza[a], Shaheen Faizi[b]

[a] *PCSIR Laboratories Complex, Off University Road, Karachi-75280, Pakistan*

[b] *H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270*

(Received 29 February 1997; accepted 30 July 1997)

Condensation reaction of (**IS**)-2(α-hydroxy ethyl) benzimidazole (**I**), 2-hydroxymethyl benzimidazole (**II**) and 1,2,3 benzotriazole (**III**) with pyrimidine *viz:* 2-aminopyrimidine (**IV-a**) and 4, 6-dimethyl-pyrimidine (**IV-b**) under the condition of Mannich reaction afforded the hitherto unreported substituted 1-aminomethyl (hydroxyalkyl) benzimidazoles and 1-aminomethyl benzotriazoles which were assigned the structures (**Va-d**) and (**VIa-b**) on the basis of their IR, ^{1}H-NMR and HREIMS spectral studies. Antibacterial activity was also evaluated.

Key words: Antibacterial activity, Mannich bases, Hydroxybenzimidazole.

Introduction

Benzimidazole derivatives are reported to possess effective biological activities (Sengupta and Goyal 1983; Yukiyoshi *et al* 1996). Recently they are evaluated for the treatment of heart failure and as cardiotonics (Hiroaki *et al* 1996). Similarly some pyrolbenzimidazoles are reported as modulators for GABAA receptors(Lene and Frank 1996), while some benzimidazole derivatives as monocationic and dicationic analogues of Hoechst-33258 are also described (Agnieszka *et al* 1996). The adduct of benzotriazole with amine and formaldehyde are also employed as intermediate for the synthesis of compounds (Katritzky *et al* 1989) due to the ease with which the benzotriazolyl moiety is displaced. The role of the pyrimidine in the biological system is evident from the fact that it is an important constituent of DNA and RNA (Vishnu Ram *et al* 1984). Diaryl pyrimidine derivatives are reported as neovascularization inhibitor and psychotropic drugs (Yasuo *et al* 1995).

Previously (Shabeen *et al* 1987 and 1990) we reported the synthesis of (*IS*)-4-aryl-4, 5-dihydroxymethyl-1H, 3H-[1,3,5]-oxadiazepion [5,6-α] benzimidazoles through intramolecular Mannich reaction of (*IS*)-2-(α-hydroxy ethyl) benzimidazole and 1-amino methyl benzotriazole from 1,2,3 benzotriazole with formaldehyde and primary aromatic amines. In the present studies aminopyrimidines were employed in place of aromaticamines.

Experimental

Melting points were taken on a Buchi 510 melting point apparatus and are uncorrected. The IR spectra were measured on a JASCO A-302 spectrophotometer. The ^{1}H-NMR were recorded in $CdCl_3$ and DMSO d_6 Brucker AM-300 ASPECT-3000 Spectrometer using TMS as internal reference. Mass spectra were taken on Finningen Mat 112-5.

General Procedure for the Preparation of (**Va-d**) *and* (**VIa-b**)*:* 2-(α-Hydroxyethyl) benzimidazole (**I**), 5-chloro-(2-hydroxymethyl) benzimidazol (**II**) and 1,2,3 benzotriazole (**III**) (0.01 mol) were dissolved in boiling ethanol (25 ml). Formaline (2 ml, 37%) and amino pyrimidine (**IVa-b**) (0.01 mol) acetic acid (0.5 ml) were added to it with good stirring. After the addition was over, refluxing continued for another two hours and then left overnight at room temperature. The solvent was removed *in vacuo* and gave (**Va-d**) and (**VIa-b**).

1[2-Pyrimidino-]-Aminomethyl-2-(α-Hydroxyethyl) Benzimidazole (V-a): White solid from EtOAc (48.8%, m.p. 156-7°C. $[\alpha]_D^{25}$+20 (c, 0.3; pyridine) υ_{max} 3400 (OH) 3250 (NH) 1610, 1580 (aromatic) cm^{-1}. Mass: m/z (rel.int.) 269 (M^+2), 238 (5), 214 (12), 174 (7), 160 (100), 147 (63), 119 (28), 109 (76), 80 (25) and 65 (24). Peak matching m/z 269.12824 ($C_{14}H_{15}N_5O$, calculated 269.12931) ^{1}H-NMR (Table 1).

1[2-Pyrimidino-]-Aminomethyl-2-(α-Hydroxyethyl) Benzimidazole (V-b): White solid from EtOAc/Pet.ether (42%) m.p. 107-108°C $[\alpha]_D^{25}$ -57.14 (c, 1.12; pyridine) υ_{max} 3380 (OH) 3250 (NH) 1600, 1580 (aromatic) cm^{-1}. Mass m/z (rel.int.) 297 (M^+,2) 278 (1), 162 (14), 146 (17), 136 (100), 119 (28), 107 (26), 93 (8), 83 (9) and 67 (27). Peak matching m/z 297.16213 ($C_{16}H_{19}N_5O$, calculated, 297.15895) ^{1}H-NMR (Table 1).

1[2-Pyrimidino]-Aminomethyl-5-Chloro-2-(Hydroxymethyl) Benzimidazole V-c): Brownish white solid from EtOAc, m.p = 160°C. (40%), υ_{max} 3390 (OH) 3250

*Author for correspondence

(NH) 1620, 1580 (aromatic) cm^{-1}. Mass m/z (rel.int) 286. (M^+, 7)258 (6) 215 (11), 184 (32), 183 (13), 182 (100), 163 (23), 164 (10), 165 (8), 153 (16), 110 (13), 108 (75) and 80 (29). Peak matching m/z 289.0739 ($C_{13}H_{12}ClN_5O$, calculated 289.074031) ^{1}H-NMR (Table 1).

1[2-Pyrimidino]-Aminomethyl-4, 6-Dimethyl 5-Chloro-2-(Hydroxymethyl) Benzimidazole (V-d): Off white solid from EtOAc m.p. 236-238°C (30%) υ_{max} 3300 (OH), 3240 (NH) 1610, 1560cm^{-1} (aromatic). Mass m/z (rel. int) 317 (M^+,1), 184 (32), 182 (100), 162 (51), 153 (39), 138 (21), 117 (17) and 90 (18). Peak matching m/z 317.102312 ($C_{15}H_{16}ClN_5O$, calculated 317.1043311 ^{1}H-NMR (Table-1).

1[2-Pyrimidino]-Aminomethyl-1,2,3 Benzotriazole (VI-a): White fluffy solid, crystallized with EtOH m.p. =157-158°C (48%). IR υ_{max} 3200 (-NH) 1600 and 1590 cm^{-1} (aromatic). Mass m/z (rel. int) 226 (M^+ 18), 198 (23), 197 (21), 167 (11) 119.5 (33), 108 (100), 97 (13) and 91 (18). Peak matching m/z 226.095708 ($C_{11}H_{10}N_6$, calculated 256.096689) ^{1}H-NMR (Table 1).

1[2-pyrimidino]-Aminomethyl-4,6-Dimethyl-1,2,3 Benzotriazole (VI-b): White amorphous powder crystallized from EtOAc. m.p. 219-22°C (50%). IR υ_{max} 3195 (NH), 1600

Table 1

^{1}H NMR chemical shift values for compounds *Va-d & VIa-b*

S. No.	Compound	CH_3 δ	CH_2 δ/Hz	CH δ/Hz	Protons of Pyrimidine ring δ/Hz	Aromatic Protons δ/Hz	Imino Protons δ/Hz*	Hydroxyl Protons δ/Hz*
1.	V-a**	1.58(3H, d) J=6.50	5.85, 6.05 2H,q) J_{gem}=14.4 J=6.72	5.45(1H, q) J=6.50	7.13(3H, m)	7.48(2H,m) 7.63(2H, m)	6.85(1H,NH,t) J=7.2	2.06(1H,s)
2.	V-b**	1.78(2H, d) J=6.57 2.24(6H, s)	6.05, 5.83 (2H, q) J_{gem}=14.7 J=6.72	5.45 (1H, q) J=6.50	6.36 (1H, s)	7.19(4H, m)	6.48(1H, NH,t) J=6.72	2.35(1H,s)
3.	V-c	-	5.07 (2H,broad s) 5.79 (2H, d) J=6.65	-	8.35(2H, d) J=4.8, 6.70 (1H, t) J=4.8	8.05 (1H, d) J=1.50, 7.55 (1H, d) J=8.5 7.17 (1H, dd) J=8.5, 1.5	8.35(1H, t) J=6.65	5.01(1H, s)
4.	V-d	2.20(3H,s) 3.29(3H,s)	5.03 (2H, broad s) 5.75(2H, d) J=4.0	-	6.45(1H, s)	7.25(1H,dd) J=2.06, 8.6 7.57(1H, d) J=2.04, 7.95 (1H, d) J=8.6	8.13 (1H, NH, t) J=4.0	5.68(1H,s) -
5.	VI-a	-	6.33(2H, d) J=6.92	-	6.68(1H,d) J=4.8, 8.33 (2H,d), J=4.8	7.97(1H, d) J=8.35, 7.9 (1H, d) J=8.36, 7.41 (1H, t)J=8.0 7.30 (1H, t) J=8.0	7.48(1H, t)	-
6.	VI-b	2.33(6H,s)	6.33(2H,d) J=7.1	-	6.46(1H,s)	7.99(2H,d) J=8.3, 7.43 (1H, m), 7.30, (1H, m)	6.58(1H, NH)	-

*Disappeared on addition of D_2O; **Recorded on 300 MHz and remaining at 400 MHz; s singlet; d doublet; dd double doublet; q quartet; t triplet.

1590 (aromatic) cm^{-1}. Mass m/z (rel. int.) 204 (M^+ 14), 226 (25), 135 (30) 136 (100), 113 (41), 108 (39), 107 (49), 93 (57), 77 (73) and 67 (81) ^{1}H-NMR (Table 1).

Evaluation of Antibacterial Activity: Compounds (**Va-d**) and (**VIa-b**) were tested for their antibacterial activity against both Gram -ve and Gram +ve bacteria namely, *S. typhi, E. coli, Kl. pneumoniae, B. bronchiseptica, Sh. dysenteriae,Sh. flexnerii, Sh. sonnei, Pseudomonas sp., Aeromonas sp.,S. typhi Para A* (Gram -ve), *S. aureus, B. pumilus, and L. plantarum* (Gram + ve). The cultures of bacteria grown over night at 37°C were used for testing antibacterial activity. The assay was carried out by overlay agar method (Lene and Frank 1996).

The plates were formed with two different media.The thick hard layer or underlay was made with antibiotic assay medium (base agar). The overlay was made with antibiotic assay medium (seed agar). First the underlay was formed by pouring base agar (melted) plates. When this layer became hard, the seed agar tubes (melted, not too hot to kill the bacteria) were inoculated with organisms and poured on sterile hardlay or underlay. The plates were kept at room temperature to get hard. Holes were bored in the medium with the help of sterilized borers. Different dilutions of the testing compounds were poured in each hole. The compounds were dissolved in DMF and blank experiment with pure DMF was also performed. Two antibiotics Ampiclox and Oxytetracycline were also used for comparison. Results are reported as zone of inhibition after 24 to 48 hours of growth at 37°C (Table 2).

Results and Discussion

In the present studies, condensation of (I*S*)-2-(α-hydroxyethyl) benzimidazol (**I**), 2-(hydroxymethyl) benzimidazole (**II**) and 1,2,3 benzotriazole (**III**), formaline and 2-amino pyrimidines (**IVa-b**) under Mannich reaction condition afforded the hitherto unreported 1-aminomethyl-pyrimidino 2-(α-hydroxyethyl) benzimidazole (**V-a**), 1-aminomethyl-4, 6-dimethyl pyrimidino-2-(α–hydroxyethyl) benzimidazole (**V-b**), 1-aminomethyl-pyrimidino-2-hydroxy methyl, benzimidazole (**V-c**), 1-aminomethyl-4, 6-dimethyl pyrimidino 2-(hydroxymethyl) benzimidazole (**V-d**), 1-aminomethyl pyrimidino 1,2,3 benzotriazole (**VI-a**) and 1-amino methyl-4, 6-dimethyl pyrimidino-2-(hydroxymethyl) benzimidazole (**VI-b**) Scheme-I. The structures of these compounds were established with the help of spectroscopic studies.

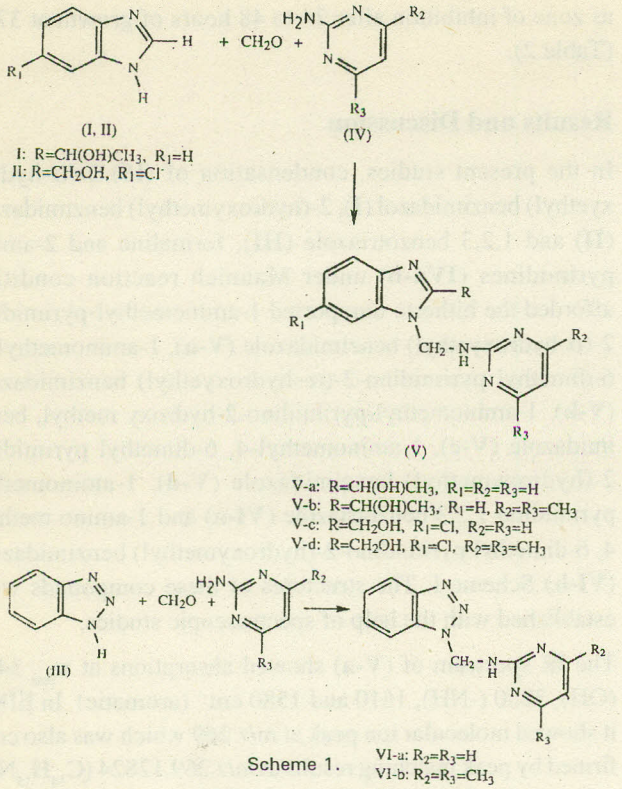
The IR spectrum of (**V-a**) showed absorptions at υ_{max} 3400 (OH), 3250 (-NH), 1610 and 1580 cm^{-1} (aromatic). In EIMS it showed molecular ion peak at m/z 269 which was also confirmed by peak matching results at m/z 269.12824 ($C_{14}H_{15}N_5O$ calculated as 269.1293). In the ^{1}H NMR spectrum of (**V-a**) a three proton doublet at δ 1.58 (J=6.50 Hz) indicated the presence of a methyl group while a quarter centred at δ 5.45 (J=6.50 Hz) was assigned to the methine proton. Also a singlet due to OH group appeared at δ 2.06 which disappeared

Table 2

Antibacterial activity (zone of Inhibition in mm)

S. No.	Name of Organism	V-a	V-b	V-c	V-d	VI-a	VI-b	Ampi clox	Oxytetra cycline
	Gram Negative								
1.	*S.typhi*	19	25	20	22	21	20	18.5	15
2.	*E. coli*	20	20	-	-	25	22	18.5	15
3.	*Kl. pneumoniae*	23	20	22	23	25	23	17.4	18
4.	*B.bronchiseptica*	25	25	28	28	26	25	-	-
5.	*Sh. dysenteriae*	20	21	20	24	23	21	18.1	16
6.	*Sh. flexnerii*	32	28	24	25	-	21	18.2	18
7.	*Sh. sonnei*	22	26	20	24	21	22	18	18
8.	*Pseudomonas sp.*	20	18	-	20	-	17	20.8	17
9.	*Aeromonas sp.*	21	-	19	20	-	22	18.0	17
10.	*S. typhi* Para A	24	21	21	24	-	21	18.3	16
	Gram Positive								
1.	*S. aureus*	15	19	15	15	20	18	22.2	11
2.	*B.pumilus*	15	15	19	13	-	16	18.1	12
3.	*L.plantarum*	16	16	18	15	19	-	20.2	11

Conc. =10 mg ml^{-1}, DMF; -ve no inhibition diameter of the well = 8.2 mm.



Scheme 1.

on addition of D_2O. These results indicated the presence of a H-C(OH)-CH_3 in (**V-a**). Two split quartets at δ 5.85 and δ 6.05 (J_{gem} 14.4 Hz) were assigned to the geminal protons of H-C-H group while a triplet centred at δ 6.85 (J=7.2 Hz), which disappeared on addition of D_2O was ascribed to NH protons.

The chemical shift of NH protons showing vicinal coupling with the proton of H-C-H suggested the presence of N-CH_2-

Table 3

^{13}C-NMR chemical shift assignments and corresponding δ value of protons by HMQC of V-c

S.No.	Carbon No.	δ for Carbon	δ for Proton
1.	C-2	155.46	-
2.	C-4	119.97	7.55
3.	C-4a	135.40	-
4.	C-5	121.72	7.17
5.	C-6	126.62	-
6.	C-7	111.35	8.05
7.	C-7a	140.75	-
8.	C-8	50.10	5.79
9.	C-9	160.96	-
10.	C-10	158.06	8.35
11.	C-11	111.83	6.70
12.	C-12	157.00	8.35
13.	C-13	56.61	5.07

HMQC of V-c

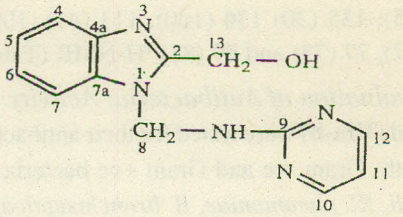


Fig. 1.

group which indicated that N-Mannich condensation has occured. In the down field region multiplet centred at δ 7.13 was assigned to the 3-protons of the pyrimidine ring while the aromatic protons appeared as two multiplets at δ 7.48 (2H) and δ 7.63 (2H). The EIMS of compound (**V-b**) showed molecular ion peak at m/z 297 which was also confirmed by peak matching results at m/z 297.15895 ($C_{16}H_{19}N_5O$) calculated as 297.16213. The ^{1}H-NMR spectra of (**V-b**) showed similar pattern as (**V-a**) except an additional six proton singlet (2 x CH_3 groups) at δ 2.24 due to the presence of methyl groups on pyrimidine ring.

The IR spectrum of (**Vc-d**) showed absorption bands between υ_{max} 3390-3200 cm^{-1} characteristic for (-OH) and (NH) along with peaks between 1505-1601 due to the aromatic ring. The mass spectra gave the molecular ion peak at m/e 289, corresponding to the molecular formula $C_{13}H_{12}ClN_5O$. It was confirmed by its peak matching results at m/z 289.069028 ($C_{13}H_{12}ClN_5O$;calculated 289.074031).

The ^{1}H-NMR spectrum of (**V-c**) exhibited a doublet δ 5.79 (2H, J=6.65) and a singlet at δ 5.07 for the two methylene groups as CH_2-NH a CH_2-OH present in the molecule. The triplet at δ 8.35 (J=6.65) indicated the imino group which disappeared on addition of D_2O and the doublet at δ 5.79 became singlet, this confirmed the -CH_2-NH- moiety in the molecule. In the down field regions doublet and a triplet at δ 8.35 (J=4.8 Hz) and δ 6.7 (J=4.8 Hz) were assigned to the three protons of the pyrimidino ring. The aromatic protons were distinguished at two doublets and a doublet of doublet at δ 8.05 (J=1.50 Hz) δ 7.53 (J=8.5 Hz) and δ 7.17 (J=8.5 Hz, 1.5 Hz) respectively. While singlet at δ 5.01 was due to (OH) group which disappeared on addition of D_2O (Table 1).

The carbon ^{13}C-NMR of (**V-c**) was assigned on the bases of DEPT and 2D one-bond ^{13}C/^{1}H chemical shift correlation experiments and gave result consistent with structure of (**Vc**). The ^{13}C-NMR spectrum d_6-DMSO 100 Mz indicated the presence of 13 carbon atoms. The peaks at δ 50.12 and δ 56.61 were assigned to the two methylene carbon on CH_2-NH and CH_2-OH. The three carbons of pyrimidine ring appeared at δ 158, δ 158.0 and δ 111.83 respectively. The four qarternary carbon atoms present in the molecule were

assigned at δ 135.40, 140.75, 155.46 and 160.96 respectively. While the remaining four carbon atoms belonging to benzimidazole moiety were shown at δ 111.35, 119.97, 121.72 and 126.62. The ^{13}C-NMR data has been shown in Table 3 and Fig.1.

The mass spectrum of (**V-d**) showed molecular ion at m/z 317 coresponding to its molecular formula $C_{15}H_{16}ClN_5O$ which was also confirmed by its peak matching result at m/z 317.102312 ($C_{15}H_{16}ClN_5O$, calculated 317.1043311). In the ^{1}H-NMR spectra of (**V-d**), the chemical shifts obtained for methylene, aromatic and pyrimidino protons showed identical pattern to (**V-c**) except two sharp singlets centred at δ 2.20 and 3.29 for two methyl groups indicative of their presence at the pyrimidino ring.

The structure of Mannich bases of 1,2,3 benzotriazole (**VIa-b**) has also been established by spectroscopic techniques. The IR spectrum of (**VIa-b**) indicated characteristic absorption between 3200-3250 cm^{-1} which was consistent with the presence of NH group. The molecular spectrum of (**VIa-b**) afforded the molecular ion peak at m/z 226 and m/z 254, corresponding to the molecular formula $C_{11}H_{10}N_6$ and $C_{13}H_{14}N_{6,}$ respectively. Exact mass measurement on the molecular ion peak of each afforded the mass 226.0945775 and 254.125978 (calculated for $C_{11}H_{10}N_6$, 226.095768; for $C_{13}H_{14}N_6$ 254.127988). The base peaks of these compounds were present at m/z 108 and m/z 136 respectively.

In the ^{1}H-NMR spectrum of (**VI-a**), a doublet centred at δ 6.33 (J=6.9 Hz) and a broad hump at δ 7.48 attributed to -NH-CH_2-. In case of (**VI-b**) chemical shifts for imino and methylene protons were found very close to that recorded for (**VI-a**). Moreover, the chemical shifts for aromatic and pyrimidino rings of (**VI-b**) were similar to (**VI-a**); however, a six proton singlet at δ 2.33 in (**VI-b**) confirmed the presence of a dimethyl pyrimidine moiety in (**VI-b**) (Table 1).

The compounds thus synthesised were subjected for their antibacterial activity by using overlay agar method (Crickshank *et al* 1975) against *S. typhi, E. coli, Kl. pneumoniae, B. bronchiseptica, Sh. dysenteriae, Sh. flexnerii, Sh. sonnei, Pseudomonas sp., Aeromonas sp., S. typhi* Para A. (Gram -ve), *S. aureus, B. pumilus and L. plantarum* (Gram +ve). The results shown in Table 2 reveals that these compounds except (**VI-a**) are as active as Ampiclox and Oxytetracycline against Gram-ve organisms.

References

Agnieszka C, Wilson W D, Boykin D W 1996 Synthesis of mono-cationic and dicationic analogs of Hoecl. *Heterocyclic J Chem.* **33,** 1393-97.

Crickshank R, Dugid J P, Marmion D P, Swain R H A 1975 *Medical Microbiology,* **12th** eds (Churchill-Living Stone Edin-burgh, London.

Hiroaki Y, Yukiko G, Masako K, Toshihias S, Massanori S, Noryoshi S, 1996 *Preparation of n-(2-pyridyl)-1-('2-tetrazolo-5-ylbisphen-4-yl-H-benzimidazole-2-carboxamide derivatives as angiotensin* II *antagonists.* Jp. Patent of CA **125** 275 884m.

Katritzky A R, Yannakopoulou K, Rasala P L D, Urogdi L, 1989 The Chemistry of n-substituted benzatriazoles. Part XIV. Nova routes to secondary and tertiary imines and to N-N-disubsituted hydroxylamines. *J Chem Soc Perkin Trans* **1,** 225.

Lene T, Frank W, 1996 Preparation of pyrrolylbenzimidazoles as GABAa receptor modulators PCT Int A ppl. WO **96** 33 192.

Sengupta A K, Goyal M J 1983 Synthesis and biological screening of some oxadiazolyl/triazolyl/thiadiazolyl and benzimidazoles. *Indian Chem. Soc* **60,** 766.

Shaheen A H, Najma M and Qureshi I H 1987 Potential andtibacterial agents. part-I. Synthesis of substituted 1-arylamino methyl benzotriazoles. *Pak j sci ind res* **30** (2) 97.

Shaheen A H, Tahira B S, Naheed S, Najma M, Qureshi I H 1990 Studies on intramolecular Mannich reaction of (S)-2 (α-hydroxyethyl) benzimidazole, synthesis of (IS)-4-aryl-4, 5-dihydro-1-methyl-1H, 3H, [1, 3, 5]-oxadia-zepino [5, 6-a] benzimidazoles. A new class of heterocyclic compounds. *Heterocycles* **31** (7), 1245.

Vishnu R, Vanden B D A, Vliethick A J, 1984 5-cyno-6-aryluracil and 2-thiouracil derivatives as potential chemotherapeutic agents. *J Het Chem* **21,** 1307.

Yasuo K J, Kenji N, Shozo Y, Tetsuji A, Takashi S, Masayasu K, 1995 *Preparation of diarylpyrimidine derivatives as neovascularization inhibitors.* Int Appl WO. 9632384 (Cl CO7D 239/92).

Yu Kiyoshi A, Masaru S, Nobuyuki T, Kobayashi K K, Michihiro 1996 Synthesis of cholecystokinin. A receptor inhibitory activities of benzimidazole derivatives. *Yakugaku Zasshi* **116** (9) 735-747.