Original Research Article

(1H-Pyrrolo[2,3-b]pyridine)7-azaindole derivatives and their antiurease, phosphodiesterase and β-glucuronidase activity

Abstract: A variety of 7-azaindole analogs **1**-**14** with variable substituents on phenyl ring of phenacyl moiety were synthesized and evaluate for their urease, phosphodiesterase and β-glucuronidase Inhibitory potential. Compound **9** (IC_{50} = 2.19±0.37μM) showed potent urease inhibitory potential than standard thiourea (IC_{50} = 21.00±0.01μM). However, while compounds **10** (IC_{50} = 255.1±6.62μM) and **8** (IC_{50} = 133.3±0.46 μM), exhibited moderate urease potential. Moreover, compound **2** (IC_{50} = 20.83± 0.234uM) showed potent phosphodiesterase inhibitory potential than standard EDTA (IC_{50} = 274±0.007uM). Compound **8** (IC_{50} 192.6 ± 3.53 μM) was found to be moderate β-glucuronidase inhibitor, as compare to standard 1,4,lactone D saccharic acid (IC_{50} = 48.4 ±1.24 uM). Nevertheless, compounds 1**3** **(**36.8 % inhibition) and 1**4** **(**47.1% inhibition) showed less than 50% β-glucuronidase inhibition, therefore they were not further evaluated for their IC_{50} values. The size of the substituent, electron donating or withdrawing affect of substituents as well as the position of substituent on phenyl affects the activity

Keywords: 7-Azaindole, Urease, β-glucuronidase Inhibition, synthesis, Phosphodiesterase,

Abbreviations: *Thin layer chromatography, TLC; VDCs, voltage dependent Ca21 channels, Phosphodiesterases-I, PDE;*

Introduction:

7-Azaindoles are the most widely studied nitrogen analogue of the indole ring system [1]. The 7-azaindole nucleus has proved to be an important model for working on its own synthesis and synthesizing its different analogues [2-5]. In the past, indole derivatives have attracted the interest of scientists for their potential biological activities like [6-10] analgesic, blood pressure lowering, anti-inflammatory activities and acting as effective coronary vasodilator, cardiovascular and potent hypotensive agent [11-14]. Saify *et.al.* synthesized a series of 7-azaindole derivatives having important antimicrobial response against gram positive and gram negative bacteria [15-18]. It has been used as an indole bioisostere to improve physicochemical and pharmacokinetic properties of several drug candidates such as dopamine D4 ligands [19]. It is also an emerging pharmacophore in ATP competitive kinase inhibitors as it contains the typical motif to dock into the adenine binding pocket [20, 21].

Activities of ureases have been shown to be an important virulence determinant in the pathogenesis of many clinical conditions. Urease is directly involved in the formation of infectious stones and contributes to the pathogenesis of urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma, urinary catheter encrustation [22]. It plays an important role in the pathogenesis of gastric and peptic ulcer including cancer [23]. In agriculture, high urease activity causes significant environmental problems by releasing large amount of ammonia into the atmosphere. [24]. Therefore strategies based on urease inhibition are now considered as the first line of treatment for infections caused by urease producing bacteria [25].

β-Glucuronidase is an exoglycosidase enzyme that catalyzes the cleavage of glucuronosyl-*O*-bonds. Liver damage causes an increase in this enzyme level in blood. Liver cancer is also suspected to be related to the over expression of this enzyme. Many *β*-glucuronidase inhibitor such as 8-hydroxytriceine glucuronide, scoparic acid A and C have already been isolated from different plants and some are used clinically [26-28].

Phosphodiesterases-I (PDE) are a type hydrolases that act on di-esters of phosphoric acid and releases nucleoside-5-monophosphate from various nucleosides diphosphate and triphosphate bonds. Phosphodiesterases are widely distributed in mammalian intestinal mucosa, liver cells, serum, and snake venom, as well as in various plants [29]. PDE inhibitors have been identified as new potential therapeutic agent in pulmonary arterial hypertension, coronary heart disease, dementia, depression and schizophrenia.

Our research group is working on drug discovery, and affianced in the search of new enzyme inhibitors since a decade [13-17, 30, 32, 33]. We have recently reported 7-aza-indoles, as an excellent anaphylactic inhibitors [31]. In view of the biological significance of 7-aza-indole and its structural resemblance with compounds which were found in enzyme inhibitors. It was of immense interest to synthesize and evaluate new derivatives of 7-aza-indole against urease, *β*-glucuronidase and phosphodiesterase enzyme [33]. Herein, we describe a synthesis of derivatives of 1H pyrrolo[2,3-b] pyridine (**1-14**). Compounds **1-14** were evaluated for their inhibition against urease, *β*-glucuronidase and phosphodiesterase enzyme as well as structure-activity relationship is also described.

Materials and methods

Experimental

General procedure

Reagents were procured from Aldrich Chemical Company. The entire solvents were reagents grade. Reactions were monitored by thin layer chromatography TLC using pre-coated silica gel, GF-254 and analytical TLC was executed on silica gel (Kieselgel 60, 254, E. Merck) pre-coated 0.25 mm plates. Visualization was accomplished with ultraviolet light at 254 and 365 nm UVP UVLS-26 Series (Cambridge). Iodine vapors were also employed for the recognition of spots. All melting points were recorded on Gallenkamp melting point apparatus and were corrected. Silica granules from E. Merck was used for drying reaction product after workup. Ultraviolet spectra were recorded in methanol on a Hitachi U-3200 spectrophotometer. Infra Red spectra were measured on a Shimadzu IR 460 spectrophotometer using KBR disc. Mass spectra were determined on Varian Massen spectrometer MAT 311A spectrometer. Nuclear magnetic resonance spectra were recorded in DMSO-D_6 and MeOD on AVANCE AV 300 spectrometer operating at 300, 400, and 500 MHz.

Phosphodiesterase enzyme activity was monitored spectrophotometrically at 37°C on a microtiter plate spectrophotometer (Spectra Max, Molecular Devices).

β-Glucuronidase (E.C. 3.2.1.31) from *E. coli*, (*p*-nitrophenyl-β-D-glucuronide) was obtained from Sigma Chemical Co. Other reagents were also purchased from commercial sources.

Phosphodiesterase from both ropsatrox crude dried venom (P4631), substrate bis (p-nitrophenyl) phosphate sodium salt (N-3002), and magnesium acetate were purchased from Sigma, while ammonium acetate was purchased from Merck. Tris buffer was a product from Scharlau (TR 0423). The standard inhibitor EDTA was purchased from Avon Chem limited.

General method for preparation of compounds

7-azaindole derivatives were synthesized by refluxing equimolar quantity of 7-azaindole in acetone (20-25 ml) and was added successively and 2-bromo-2-acetonapthone, 1-adementile-

bromo ethyl-ketone, 2-bromo-3-nitro-acetophenone, 2-bromo-4-nitro-acetophenone were dissolved separately in 20-25 ml of acetone. Reaction mixture was stirred for 24 hours at 52-54°C. The process of reaction was monitored through thin layer chromatography. The crude solid product was filtered and washed with acetone. The product thus obtained was purified through re-crystallization by using ethanol and ether. The pure compound was dried in desicators over anhydrous calcium sulphate. Melting point and spectral data were obtained to confirm the structure of compound.

β-Glucuronidase Enzyme Inhibition

β-Glucuronidase activity was determined by measuring the absorbance at 405 nm of *p*-nitrophenol formed from the substrate by the spectrophotometric method. The total reaction volume is 250μl. The reaction mixture contained 185μl of 0.1 M acetate buffer, 5μl of test compound solution, 10μl of enzyme solution incubated at 37 °C for 30 min. The plates were read on a multiplate reader (SpectraMax plus 384) at 405 nm after the addition of 50 μl of 0.4*m*M *p*-nitrophenyl-β-D-glucuronide. All assays were run in triplicate [34]. The IC_{50} values were calculated using the EZ-Fit Enzyme Kinetics program, Perrella Scientific Inc., Amherst, U.S.A.

Urease Enzyme Inhibition

Reaction mixtures comprising 1 unit of urease enzyme (*Bacillus pasteurii or Jack bean*) solution and 55μl of buffers containing 100*m*M urea were incubated with 5μl of test compounds (1*m*M concentration) at 30°C for 15 min in 96-well plates.

Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn [35]. Briefly, 45μl each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70μl of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200μl. The results (change in absorbance per min) were processed by using Soft- Max Pro software (Molecular Device, USA). All reactions were performed in triplicate. All the assays were performed at pH 8.2 (0.01 M $K_2HPO_{4.}3H_2O$, 1*m*M

EDTA and 0.01 M $LiCl_2$). Percentage inhibitions were calculated from the formula 100-($OD_{testwell}/OD_{control}$) x 100. Thiourea was used as the standard inhibitor of urease.

Phosphodiesterase Enzyme Inhibition

Activity against the snake venom phosphodiesterase I (Sigma P 4631) (EC 3.1.4.1) was assayed by using the reported method [36] with the following modifications. 33 *m*M Tris–HCl buffer, pH 8.8, 30 *m*M Mg-acetate with 0.000742 U/well final concentrations using microtiter plate assay and 0.33 *m*M bis-(p-nitro phenyl) phosphate (Sigma N-3002) as a substrate. Cysteine and EDTA (E. Merck) were used as positive controls (IC_{50} = 748 lM ± 0.15, 274 lM ± 0.07, respectively). After 30 min of incubation, the enzyme activity was monitored spectrophotometrically at 37°C on a microtiter plate spectrophotometer (Spectra Max, Molecular Devices) by following the release of *p*-nitrophenol from *p*-nitrophenyl phosphate at 410*n*m. All the reactions were performed in triplicate and the initial rates were measured as the rate of change in OD/min (optical density/min) and used in subsequent calculations.

1H-pyrrolo[2,3-b]pyridine (7-azaindole) (**1**): White crystalline solid, EIMS *m/z* =118.14 amu, M. P. = 105-107 ^{o}C.

1-[3-(3,4-Dihydroxyphenyl)3-oxo-ethyl]-7H-pyrrolo[2,3-b]pyridine-1-ium;bromide(2): HREI MS: $C_{15}H_{13}N_2O_3Br$; UV: 289, 227 and 208. IR: 3100,1670,1595 and 1290, EIMS *m/z* (rel. int %): M-1($C_{15}H_{12}N_2O_3$), 269 (2), 239 (14), 193 (3), 165 (12), 137 (15), 118 (100) and 109 (25).^{1}H-NMR (D_2O, 300 MHz) δ: 8.72 (1H, d, *J* = 7.85 Hz, H-6), 8.24 (1H, d, *J* = 6.80 Hz, H-4), 7.70 (1H, dd, *J* = 8.46, 2.21 Hz, H-6), 7.67 (1H, d, *J* = 3.65 Hz, H-2), 7.62 (1H, d, *J* = 7.85, 6.80 Hz, H-5), 7.55 (1H, d, *J* = 2.19 Hz, H-2), 7.06 (1H, d, *J* = 8.46 Hz, H-5'), 6.97 (1H-d, *J* =3.65 Hz, H-3), 6.48 (2H, *s*, N-CH_2), 4.83 (1H,br-*s*, OH), 4.65 (1H, br-*s*, OH).

7-[2-(3-Nitrophenyl)-2-oxo-ethyl]-1H-pyrrolo[2,3-*b*]pyridinium;bromide (**3**): Yield =80%, HREI MS: = $C_{15}H_{12}N_3O_3Br$, EI MS: *m/z* =362.178 amu, M. P. = 224 + 2°C, UV λ_{max} (MeOH) *nm* = 299.6, 228, 200 nm, IR ν_{max} (KBr) cm^{-1} = 3232, 2916, 1965, 1797, 1697, 1529, 1475, 1356, 1224.9, 1172.8, 1091.5, 930, 883, 799, 598, 519, 481.2, EIMS = *m/z* (relative int., %):

282(M^+, $C_{15}H_{12}N_3O_3$, 9.8), 252(100), 236(41), 206(57), 193(6.1), 150(15.6), 131(53.5), 118(13.3), 90(6.2), 77(17.1), 63(6.6),^{1}H-NMR (MeOD, 300 MHz) δ: 8.93 (1H, s, H-6), 8.81 (1H, d, J = 7.79 Hz, H-4), 8.69 (1H, dd, J = 7.71, Hz, H-2`), 8.52 (1H, m, H-5'), 8.39 (1H, *s*, H-4`), 7.93 (1H, dd, J = 8.02, 3.35 Hz, H-6'), 7.77(1H, d, J = 3.35 Hz, H-5), 7.67 (1H, m, H-2), 7.01 (1H, d, J =3.50 Hz, H-3), 6.90 (2H, d, J = 3.57 Hz, H-2``).

7-[2-(2-Nitrophenyl)-2-oxo-ethyl]-1H-pyrrolo[2,3-*b*]pyridinium;bromide (**4**): Yield = 80%, HREI MS: = $C_{15}H_{12}N_3O_3Br$, EIMS: *m/z* = 362.178 amu, M.P. = 224 + 2°C, UV λ_{max} (MeOH) nm = 299.6, 228, 200 nm, IR ν_{max} (KBr) cm^{-1} = 3232, 2916, 1965, 1797, 1697, 1529, 1475, 1356, 1224.9, 1172.8, 1091.5, 930, 883, 799, 598, 519, 481.2, EIMS = *m/z* (relative int., %): 282(M^+, $C_{15}H_{12}N_3O_3$, 9.8), 252(100), 236(41), 206(57), 193(6.1), 150 (15.6), 131(53.5), 118 (13.3), 90(6.2), 77(17.1), 63(6.6),^{1}H-NMR (MeOD, 300 MHz) δ: 8.93 (1H, *s*, H-6), 8.81 (1H, d, J = 7.79 Hz, H-4), 8.69 (1H, dd, J = 7.71, Hz, H-2`), 8.52 (1H, m, H-5'), 8.39 (1H, s, H-4`), 7.93 (1H, dd, J = 8.02, 3.35Hz, H-6'), 7.77(1H, d, J= 3.35 Hz, H-5), 7.67 (1H, m, H-2), 7.01 (1H, d, J=3.50 Hz, H-3), 6.9 (2H, d, J = 3.57Hz, H-2``).

7-[2-(4-Nitrophenyl)-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium;bromide(**5**): Yield = 61%, HREI MS: $C_{15}H_{12}N_3O_3Br$, EIMS: *m/z* = 362.18 amu, M.P. = 252 + 2°C, UV λ_{max} (MeOH) nm: 270, 248 and 201; IR υ_{max} (KBr) cm^{-1}: 3390, 2920, 1690, 1600 and 1340, EIMS, *m/z* (relative int., %): 282 (M^+-Br, $C_{15}H_{12}N_3O_3$, 8), 164(4), 160(12), 132 (11), 122(18), 118(100) and 77(23), ^{1}H-NMR (MeOD, 300 MHz) δ: 8.81 (1H, d, J = 7.84, 1.08 Hz, H-6), 8.84 (2H, d, J = 8.02, Hz, H-3', 5'), 8.26 (1H, d, J = 8.99 Hz, H-4), 7.78 (2H, d, 8.02 Hz, H-2', 6'), 7.71 (1H, d, J =3.51 Hz, H-2), 7.68 (1H, dd, J = 8.99, 7.84 Hz, H-5), 7.02 (1H, d, J =3.51 Hz, H-3), 6.02 (2H, *s*, H-2").

7-[2-(2,4-Diflouro-phenyl)-2-oxo-ethyl)-1H-pyrrolo[2,3-b]pyridine-7-ium,bromide(**6**): HREI MS: $C_{15}H_{12}N_2OF_2Br$, UV (MeOH) nm: 298, 261 and 200, IR (KBr): 3400, 2900,1690,1600,1580 and 1450, EIMS *m/z* (%): M-1 ($C_{15}H_{11}BrF_2N_2O$), 316 (8), 286 (100), 205 (15), 182 (25), 131 (50), ^{1}H-NMR(D_2O , 300 MHz) δ: 8.04 (2H, d, J = 8.82 Hz, H-2), 7.88 (2H, d, J = 8.82 Hz, H-3,5'), 6.02 (2H, *s*, H-2"), 7.67 (1H, dd, J = 7.81, 5.27 Hz, H-5), 7.00 (1H, d, J = 3.62 Hz, H-3), 5.84 (2H, *s*, N-CH_2).

7-[2-(4-Flouro-phenyl)-2-oxo-ethyl)-1H-pyrrolo[2,3-b]pyridine-7-ium,bromide(7): HREI MS: $C_{15}H_{12}N_2OFBr$, UV (MeOH) nm: 298, 261 and 200, IR (KBr): 3400, 2900,1690,1600,1580 and 1450, EIMS *m/z* (%): M-1 ($C_{15}H_{11}BrFN_2O$), 316 (8), 286 (100), 205 (15), 182 (25) and 131 (50), ^{1}H-NMR (D_2O, 300 MHz) δ: 8.64 (1H-dd, *J* = 7.81, 1.06 Hz, H-6'), 8.04 (2H, d, *J* = 8.82 Hz, H-2',6), 7.88 (2H, d, *J* = 8.82 Hz, H-3,5'), 7.70 (1H, d, *J* = 3.62, H-2), 7.67 (1H, dd, *J* = 7.81, 5.27 Hz, H-5), 7.00 (1H, d, *J* = 3.62 Hz, H-3), 5.84 (2H, *s*, N-CH_2).

7-[2-(4-Chloro-phenyl)-2-oxo-ethyl)-1H-pyrrolo [2,3-b]pyridine-7-ium,bromide(8): HREI MS = $C_{15}H_{12}N_2OClBr$, UV (MeOH) nm: 298, 261 and 200: IR (KBr): 3400, 2900, 1690,1600,1580 and 1450, EIMS *m/z* (%): M-1 ($C_{15}H_{11}BrClN_2O$), 316 (8), 286 (100), 205 (15), 182 (25) and 131 (50), ^{1}H-NMR (D_2O) (300 MHz) δ: 8.64 (1H, dd, *J* = 7.81, 1.06 Hz, H-6'), 8.35 (1H,dd, *J* = 5.27, 1.06 Hz, H-4), 8.04 (2H, d, *J* = 8.82 Hz, H-2', 6), 7.88 (2H, d, *J* = 8.82 Hz, H-3,5'), 7.70 (1H, d, *J* = 3.62, H-2), 7.67 (1H, dd, *J* = 7.81, 5.27 Hz, H-5), 7.00 (1H, d, *J* = 3.62 Hz, H-3), 5.84 (2H, *s*, N-CH_2).

7-[2-(4-Methoxy-phenyl)-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridine-7-ium (9): Yellow needles, HREI MS: *m/z* $C_{16}H_{15}N_2O_2Br$, UV**:** 389.4, 284.8, 223.0, IR: 3449.6, 3010.8, 1680.5, 1341.5 1309.5, 1236.1 and 1101.2; EIMS *m/z* (rel. int %): 266 (M^+-Br, $C_{16}H_{15}O_2N_2$, 31), 238(55), 149(2), 135(100), 131(10), 118(6) and 107(12),^{1}H-NMR: δ 8.78-8.75 (1H, d, *J* = 7.77 Hz, H-6'), 8.45-8.43 (1H, d, *J* = 6.178 Hz, H-4), 8.14-8.11 (2H, d, *J* = 8.88 Hz, H-5), 7.73-7.72 (1H,d, *J* =3.528 Hz, H-2), 7.66-7.62 (1H, m, H-6), 7.16-7.13 (2H, d, *J* = 8.925 Hz, H-3, H-5'), 6.99-6.98 (1H, d, *J* = 3.55 Hz, H-2), 6.43-6.41 (1H, d, *J* = 5.72 Hz, H-3), 3.30-3.29 (1H, *s*, H-1).

Naphthalen-2-yl-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium,bromide(10): Yield = 86.2%, HREI MS: $C_{19}H_{15}N_2OBr$, EIMS = *m/z* (relative intensity; %)= 367.23 amu, M.P.= 267 ± 2°C, UV λ_{max} (MeOH) nm = 340, 293, 250, 224, 203, IR ν_{max} (KBr) cm^{-1} = 3755, 2923, 1687, 1361, 987, 823.5, 783, 545, 474.5; EIMS = *m/z* (relative int, %): 286 (M^+ -HBr $C_{19}H_{14}N_2O$, 7.40), 259(8.2), 258(62), 257(52), 155(15), 81(18), 77(22), ^{1}H-NMR (DMSO-d6, 300 MHz), δ 8.86 (2H, d, *J* = 7.047, H-6, 4), 8.60(1H, d, *J* = 6.09, H-5), 8.24(1H, d, *J* = 7.71, H-10'), 8.07-8.03(2H,

m, H-2', 8'), 7.96 (2H, d, *J*=3.43, H-5', 3'), 7.76(2H , m, H-6' ,7'), 6.62(2H, d , *J* =3.50, H-2', 3'), (2H, *s*, H-2'').

7-(2-Adamantan-1-yl-2-oxo-ethyl]-1H-pyrrolo [2,3-b]pyridinium, bromide(**11**); yield = 81.5 %, HREI MS: $C_{19}H_{23}N_2OBr$, EIMS: *m/z* =375.30 amu, M. P. = 287 + 2^0C, UV λ_{max} (MeOH) nm = 299.6, 226.0, IR $\nu_{\mathbf{max}}$ (KBr) cm^{-1}= 3409, 2918, 2850, 2773, 1712, 1620, 1458, 1357, 1164, 1097, 887, 779, 727, 661, 536, 476. EIMS = *m/z* (relative intensity; %), 295 (M^+, $C_{19}H_{23}N_2O$, 7.3), 294 (35), 237(2.6), 176(3), 159(15.5), 131(100), 93(18.4), 79(27),^{1}H-NMR (DMSO-d6, 300 MHz): *δ* 8.76-8.79 (1H, d, *J* = 8.68 Hz, H-6), 8.48 (1H, d, *J* = 6.15Hz, H-4), 7.95(1H, d, *J* = 3.48 Hz, H-2), 7.66 (1H, dd, *J* = 7.8, 1.5 Hz, H-5), 6.97 (1H, d, *J* =3.57, H-3), 6.06(2H, s, H-2``), 2.07(5H, *s*, H-4', 6, 7', 8', 9'), 1.97(5H, *s*, H-10', 5', 3', 2'), 1.78-1.68 (6H, m, H-3', 6', 6', 7', 9',10').

7-(3-Oxo-3-phenyl-propyl)-1H-pyrrolo[2,3-b]pyridine-7-ium,chloride(**12**): HREI MS= $C_{16}H_{15}N_2OCl$, EIMS *m/z* (%): M-1 ($C_{16}H_{14}ClN_2O$), 316 (8), 286 (100), 205 (15), 182 (25) and 131 (50). ^{1}H-NMR, D_2O (300 MHz) *δ*: 8.64 (1H-dd, *J* = 7.81, 1.06 Hz, H-3,5), 8.35 (1H, dd, *J* = 5.27, 1.06 Hz, H-4), 8.04 (2H, d, *J* = 8.82 Hz, H-2, 6), 7.88 (2H, d, *J* = 8.82 Hz, H-3',5'), 7.70 (1H, d, *J* =3.62, H-2'), 7.67 (1H, dd, *J* = 7.81, 5.27 Hz, H-5'), 7.00 (1H, d, *J* = 3.62 Hz, H-3'), 5.84 (2H, *s*, N-CH_2).

7-Phenethyl-1H-pyrrolo[2,3-b]pyridin-7-ium, bromide (**13**): UV (MeOH) nm: 390.0, 293.0, 224.0, 203.4, 198.6; IR υ_{max} (KBr) cm^{-1} 51.5, 1647.6, 1495.0, 1357.4, 1307.8 and 1189.1; EIMS (relative int, %): 223 (M^+-Br, $C_{15}H_{14}N_2$ (10), 132(3), 118(100), 104(8), 91(7), 77(6); ^{1}H-NMR (MeOD, 300 MHz): *δ* 8.63-8.61(1H, d, *J* = 7.76 Hz, H-6), 8.20-8.18 (1H, d, *J* = 6.17 Hz, H-4), 7.74-7.73 (1H, d, *J* = 3.52 Hz, H-5), 7.45-7.40 (2H, m, H-3, H-5'), 7.2-7.18 (2H, t, *J* = 6.33 Hz, H-1, H-2), 7.02-6.99 (2H, m, H-2, m, H-2, H-3), 6.93-6.92 (1H, d, *J* = 3.51 Hz, H-1), 5.02-4.97 (1H, t, *J* = 6.92 Hz, H-1"), 4.57 (1H, *s*, H-2").

7-(4-Oxo-4-thiophen-2-yl-butyl)-1H-pyrrolo[2,3-b]pyridin-7-ium; chloride (**14**): UV: 389.4, 286.2, 263.8, 222.2, 204.0; IR: 3075.8, 2469, 1496.5, 1510.5, 1359.2, 1310.9, 1246.2; EIMS: 270(M^+-Cl, $C_{15}H_{15}ON_2$, *s*, 19), 185 (2), 159(28), 132(50), 126(48), 118(100), 111 (87), 83(9);

^{1}H-NMR: δ 8.68-8.65 (1H, d, J = 7.93 Hz, H-6), 8.55-8.53 (1H, d, J = 6.32 Hz, H-4), 7.87-7.80 (1H, m, H-2, H-4'), 7.58-7.53 (1H, t, J = 7.015 Hz, H-5), 7.20-7.17 (1H, t, J = 4.38 Hz, H-3), 6.97- 6.95 (2H, d, J = 3.48 Hz, H-2, H-3), 3.3-3.2 (1H, m, H-1), 2.47 – 2.43 (2H, m, H-2', H-3').

Result and discussion

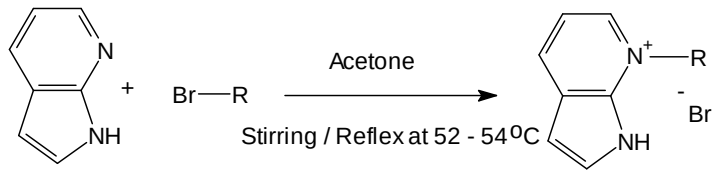
Chemistry

7-azaindole (0.01 mol) and substituted phenacyl halide (0.01 mol) were dissolved separately in acetone (20 mL) and then mixed together in a round-bottommed flask (Fig.**1**). Reflux reaction mixtures was monitored for about 24 h. The reaction completion was monitored by TLC. After completion of reaction, the precipitates were filtered, and washed with warm acetone. The obtained products were re-crystallized from methanol. The pure compounds were dried in vacuum over anhydrous calcium sulfate. The structures of synthetic derivatives were elucidated by different spectroscopic techniques including, UV, IR, EI-MS and ^{1}H-NMR. All compounds gave satisfactory elemental analysis. The compounds herein described were synthesized as a part of study complementary to β-glucuronidase, phosphodiesterase and urease inhibition investigation of fused pyrrolo pyridine system. All the 7-aza indole were prepared under the conditions recorded in experimental section (Fig.**1**).

As a result of our continued interest in 7-aza indole as potential antibacterial and cytotoxic agents [13-14,30], we now wish to report the investigation for β-glucuronidase inhibition as well as other enzyme inhibition including of 7-aza indole derivatives and their structure activity relationship. So in our present work we designed different derivatives of aza-indole in which we replaced in different groups with phenacyl chain at different position to find a selective inhibitor.

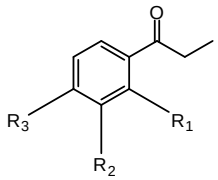
The ^{1}H-NMR spectra were specially helpful in deciding the substitution pattern at the different positions of phenacyl part of the molecule. The NMR chemical shifts of compounds **2-14** are presented in Experimental section.

The molecular ion peaks of the compounds were obtained from EIMS spectra [31]. A series of 7-azaindole derivatives were prepared by quaternization with phenethyl bromide and substituted phenacyl halides, the side chain thus possessed certain features common to those present in sympathomimetic amines and related compounds.



1 **2a-14a** **2-14**

Reactants No. R



2a R_1 = H R_2 = OH R_3 = OH

3a R_1 = H R_2 = NO_2 R_3 = H

4a R_1 = NO_2 R_2 = H R_3 = H

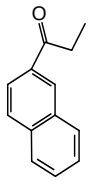
5a R_1 = H R_2 = H R_3 = NO_2

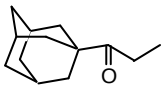
6a R_1 = F R_2 = H R_3 = F

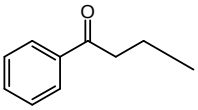
7a R_1 = H R_2 = H R_3 = F

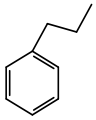
8a R_1 =H R_2 = H R_3 = Cl

9a R_1 = H R_2 = H R_3 = OCH_3

10a 

11a 

12a 

13a 

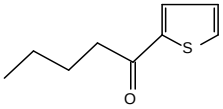
14a 

Fig.1: Synthesis of 7-azaindole derivatives **1-14**

The structures of compounds **1-14** were identified by spectroscopic studies previously reported in the literature [31].

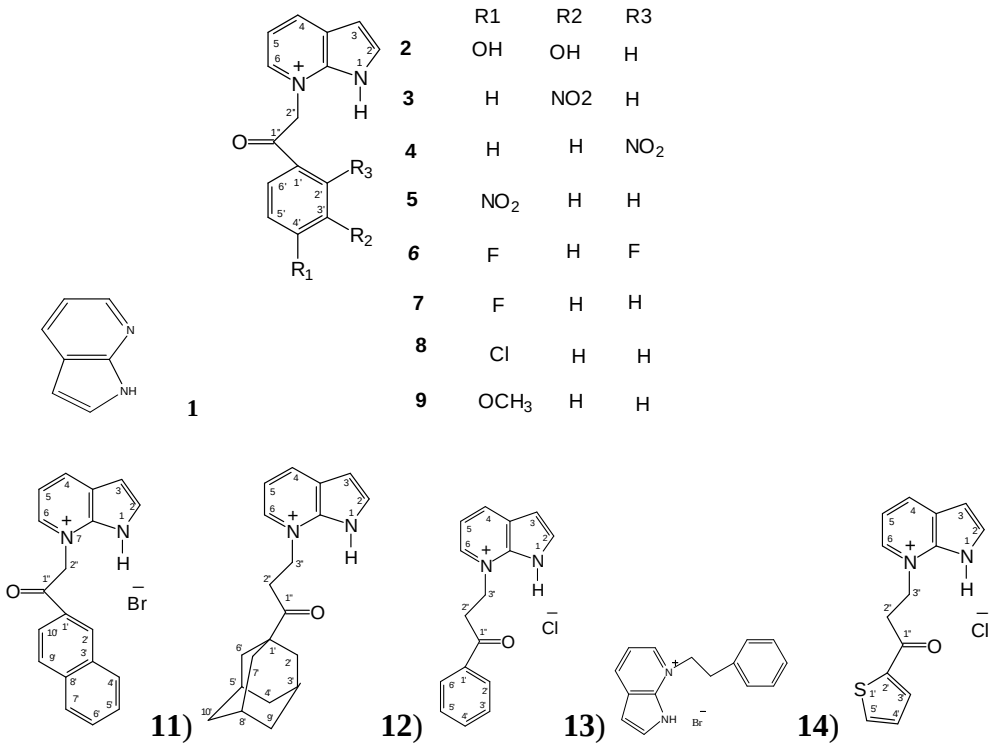


Fig.2: Structures of 7-azaindole derivatives **I -14**.

Compounds **1-14** were screened for β-glucuronidase inhibitory activity (Table-**1**). Parent compound **1** showed no activity. Among the synthesized analogues of compound **1**, compounds **2**, **8**, **9**, **10**, **11**, **13** and **14** showed reasonable % inhibition, while compounds **3-5**, **6**, **7** and **12** displayed no activity. Compound **8** (IC_{50} 192.6 ±3.53 μM), containing chloro derivative displayed maximum inhibition, while compound **14** showed 47.1 % inhibition and was the second potent β-glucuronidase inhibitor of the series. Nevertheless, naphthyl derivative **10 (**20 % inhibition), and benzyl derivative **13 (**36.8 % inhibition) showed almost same level of inhibition with 20 and 37% inhibition. The more β-glucuronidase activity of compound **8** than compounds **13** and **14** is due to electron withdrawing effect of chlorine as compared to having unsubstituted benzene and sulphur containing five membered ring moieties. The compounds 1**3** with unsubstituted benzene and **14** with sulphur containing five membered ring exhibited less activity as compared to **8** due to change in substitution group at phenacyl moiety.

7-azaindole derivatives **1-14** were screened against urease enzyme [37] according to literature protocol [38,32,33]. They showed a varying degree of urease inhibitory activity having IC_{50} values ranging between 2.19- 255.1μM, when compared with standard thiourea (IC_{50} = 21 ± 0.11μM) (Table-1).

When parent compound **1** was screened along with its newly synthesized derivatives **2-14**, it was found that parent compound showed weak urease enzyme inhibitory activity (2.9%) at 0.5 *m*M concentration. Among the synthesized analogues, compounds **2**, **6**, **11**, **12** and **13** exhibited weak activity (44.4, 41.8, 16.10, 21.8 and 6.7%) against urease enzyme, while compounds **3**, **4, 5** and **7** were found devoid of any activity. Derivatives of parent compound **1** are not making profound difference in the enzyme inhibition effect except compounds **8**, **9**, **10** (chloride, methoxy, naphthalene derivatives). These compounds were selected for IC_{50} value and these showed IC_{50} values 133.3±0.46, 2.19±0.37 and 255.1±6.62μM respectively. Compound **9** showed remarkable inhibition with an IC_{50} value of 2.19±0.37μM, which is about ten times more active than the standard drug thiourea (IC_{50} = 21± 0.11**).** Compounds **8** and **10**, with IC_{50} values of 133.3±0.46 and 255.1±6.62μM showed encouraging results.

Compound **9** (IC_{50} = 2.19±0.37*u*M) having *para* methoxy substituted phenyl ring showed potent urease inhibitory potential. However, Compound **8** having *para* chloro substituted phenyl. Compound **10** having naphthalene group instead of benzene ring. The difference in activities might be due to change in nature of substituent. The more activity of Compound **9** than Compounds **8** and **10** are due to more electron donating effect of methoxy as compared to chlorine group. The difference in activity might be due to numbers as well as nature of substituents.

Compound **9**, methoxy analogue is the most active in urease assay and the most active structure of the series in the urease assay suggesting that the methoxy group at para position of benzene ring provides a sterically-favored orientation for a fruitful and most significant interaction with enzymes. While compounds **8** and **10** having chlorine and naphthalene substitution instead of methoxy was unlikely to interfere with carbohydrates digestion and glycoprotein and hence found to be less active as compare to **9**.

As we can see that attaching the different molecules at N atom of aza-indole makes the compound less active or decreases effect. An exceptional case is the compound **9** where good activity has been observed. The structural difference between all analogues except **10** is the substitution at para position may suggest that presence of methoxy group is contributing in the activity. Comparing the halide analogues compound **8** chloro derivative showed better effect as compared to flouro derivative compound **6** (41.8 % inhibition).

In phosphodiesterase-I enzyme inhibition assay the compound **2** (IC_{50} = 20.83 $\pm$0.234*u*M) showed potent phosphodiesterase inhibitory potential than standard EDTA (IC_{50} = 274$\pm$0.007*u*M). caused by the presence of *meta*,*para* hydroxy substituted benzene ring. Among the synthesized analogues, compound **3**,**4,5,7,10** and **11** exhibited weak activity (5.6, 18.9, 6.7, 17.1, 20.6 and 17.8%) against phosphodiesterase enzyme, while compounds **6**, **8,9, 12, 13** and **14** were found devoid of any activity.

The more activity of compound **2** than the rest of the compounds is due to more electron withdrawing effect of *meta*, *para* hydroxy substituted benzene ring. The compound **4**, **10** and **11** exhibited less activity as compared to **2** by the change in substitution group at phenacyl moiety.

After analyzing the results obtained by urease, β-glucosidase and phosphodiesterase-I enzymes inhibition assay, the compound **2** is the most active compound of the series in phosphodiesterase-I enzymes inhibition assay and was completely inactive in β-glucosidase assay. Similerly the compound **9** is the most active compound of the series in urease enzymes inhibition assay and was completely inactive in phosphodiesterase-I assay. Beside this the compound **8** is the most active compound of the series in β-glucosidase enzymes inhibition assay and was completely inactive in phosphodiesterase-I assay. From this study new potent inhibitors (compound **9**, **2** and **8**) were identified with acceptable correlation between biological activities and binding energies. We also succeeded in identifying two potent selective inhibitors (compound **2** and **9**). Further studies will lead to a better understanding of structure and activity relationship of these compounds.

Biological Activity

Compound **1-14** were screened for phosphodiesterase inhibition activity. The parent compound (**I**) investigated for phosphodiesterase inhibition, showed insignificant activity. Compound **2** exhibited potent results as compared to standard EDTA.

Table – I: β- Glucuronidase, phosphodiesterase and urease inhibition activity of 7- azaindole derivatives **1-14**.

S.No	**β- Glucuronidase**		**Phosphodiesterase**		**Urease**	
	% Inhibition	IC_{50} ±SEM [a] [μM]	% Inhibition	IC_{50} ±SEM [a] [μM]	% Inhibition	IC_{50} ±SEM [a] [μM]
1	-	-	17.91	-	2.91	-
2	6.31	-	86.01	20.83±0.234	44.41	-
3	-NA[b]	-	5.61	-	- NA[b]	-
4	- NA[b]	-	18.91	-	- NA[b]	-
5	- NA[b]	-	6.71	-	- NA[b]	-
6	- NA[b]	-	- NA[b]	-	41.81	-
7	- NA[b]	-	17.11	-	- NA[b]	-
8	78.51	192.6 ± 3.53	- NA[b]	-	74.81	133.3+_0.46
9	13.61	-	- NA[b]	-	75.81	2.19± 0.37
10	20.01	-	20.6	-	66.61	255.1±6.62
11	13.01	-	17.8	-	16.10	-
12	- NA[b]	-	- NA[b]	-	21.81	-
13	36.81	-	- NA[b]	-	6.71	-
14	47.11	-	- NA[b]	-	30.91	-
Standards 1,4,Lactone D saccharic acid[C]		48.4±1.24				
EDTA[d]				274 ± 0.007		
Thiourea[e]						21± 0.11

a SEM is the standard error of the mean.
b NA–not active.
c.1,4,Lactone D saccharic acid standard inhibitor for β-Glucosidase activity
d EDTA standard inhibitor for phosphodiesterease activity.
e.Thiourea standard inhibitor for urease activity

In the bio-screening of the compounds for phosphodiesterase inhibition, compounds **4**(ortho-nitro derivative)**, 7**(Flouro derivative)**, 10** (Naphthalene derivative)**, 11**(7-(2-Adamantan-1-yl-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium bromide derivative) were found moderately active. ~~While~~ Compounds **3** and **5** showed week activity. During the course of synthesis of derivatives of aza-indole, compound **2** attracted our attention as potent phosphodiesterase inhibitor. Compound **2** showed remarkable phosphodiesterase inhibition with an IC_{50} value of 20.83±0.234μM, which is about twenty times more active than the standard drug EDTA (IC_{50} = 274±0.007μM). The result indicated that OH substitution in phenacyl ring at 3,4 position (meta, para benzene) is making significant change in the activity with IC_{50} value 20.83±0.234μM. Results of phosphodiesterase activity are presented in Table-**1.**

Investigation for β-glucuronidase inhibition activity opposite results have been observed as compared to urease inhibition. Approximately similar inhibition of β-glucuronidase inhibition and urease inhibition by compound **8** suggested that though the presence of Cl functional group imparting inhibitory activity but the position of Cl in the phenacyl ring is not important for any significant difference in the activity Table-**1**.

Compound **9** showed remarkable inhibition with an IC_{50} value of (IC_{50} = 2.19±0.37μM) , which is about ten times more active than the standard drug thiourea (IC_{50} = 21± 0.11μM**).** Compounds **8** and **10**, with IC_{50} values of 133.3±0.46 and 255.1±6.62μM showed encouraging results.

Although 7-azaindole was synthesized but the useful biological activities was unexplored for many years.There was no data available that 7-azaindole derivatives can be used as β-glucuronidase, urease and phosphodiesterase inhibitors. So we decided to do their activity. By doing so we got very promising results.

Declaration of interest

The authors report no conflicts of interest.

Conclusion

There were fourteen close derivatives of 7-azaindole and out of these three i-e 7-[2-(4-chloro-phenyl)-2-oxo-ethyl)-1H-pyrrolo [2,3-b]pyridine-7-ium,bromide (**8**), 7-[2-(4-Methoxy-phenyl)-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridine-7-ium (**9**), and Naphthalen-2-yl-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium;bromide (**10**) exhibited useful anti-urease activity**.** Compound **9** (IC_{50} = 2.19 ± 0.37uM) showed potent urease inhibitory potential than standard thiourea (IC_{50} = 21.00 ± 0.01μM). Compound 1-[3-(3,4-dihydroxyphenyl)3-oxo-ethyl]-7H-pyrrolo[2,3-b]pyridine-1-

ium;bromide (**2**) (IC_{50} = 20.83 ± 0.234*u*M) showed potent phosphodiesterase inhibitory potential than standard thiourea (IC_{50} = 21.00 ± 0.01μM). Compound **8** (IC_{50} =192.6 ± 3.53 μM) was also found to be β-glucuronidase inhibitor, as compared to standard 1,4,lactone D saccharic acid, (IC_{50} = 48.4 ±1.24*u*M). i-e 7-[2-(4-chloro-phenyl)-2-oxo-ethyl)-1H-pyrrolo [2,3-b]pyridine-7-ium,bromide (**8**). Overall results are significant and encouraging and because of their urease and phosphodiesterase inhibition potential they can be selected as a useful lead molecules. All the results of this study indicated the azaindole compound **9** as interesting as it leads for anti-urease's drug development being urease inhibitors, and compounds **2** and **8** could serve as potential lead compound with promise for further research of drug development in the field of phosphodiesterase and β-glucuronidase enzyme inhibition. The potency of these compounds, especially compounds **2**, **9** and **8** suggests that this is a new class of inhibitors as it provides a solid base for using them as lead molecules, for designing effective therapeutic agent to treat these diseases.

Present study opens-up an avenue for further research on 7-azaindole derivatives for their evaluation against urease, phosphodiesterase and β-glucuronidase enzymes. Structure activity relationship studies with the standard molecules provided justification for their activity and given better understanding of their inhibiting potential depending upon their pharmacophoric regions.

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In hope to synthesize novel potential α-Glucosidase and phosphodiesterase-I derivatives, a series of 7-Azaindoles are prepared. All synthetic derivatives (**II-14**) were screened for their *in vitro* urease, α-glucosidase and phosphodiesterase-I activities (Table-**1)**. Some preliminary conclusions concerning about the SAR of the 7-Azaindoles derivatives against α-glucosidase and phosphodiesterase-I inhibiting activity can be drawn from the data collected in table **1**.

In urease activity compounds **9** (IC_{50} = 2.19 ± 0.37*u*M showed IC_{50} value superior to the standard thiourea (IC_{50} = 21.00 ± 0.01μM) and is considered excellent inhibitor against urease. While in phosphodiesterase-I assay the compounds **2** (IC_{50} = 20.83 ± 0.234*u*M) showed remarkably

highest potent activity as compared to standard thiourea (IC_{50} = 21.00 ± 0.01μM). and thus considered excellent inhibitor against phosphodiesterase assay.

Only compound **8** showed comparable β-glucosidase activity IC_{50} 192.6 ±_3.53 μM as compared to standard (1,4,lactone D saccharic acid, IC_{50} = 48.4 ± 1.24 *u*M). They are potential molecules for the development of a new class of phosphodiesterase-I, urease and α-glucosidase inhibitors.

In α-glucosidase inhibition assay the compound **IV** (IC_{50} = 101 ± 6.7 *u*M) ~~showed remarkably highest inhibitory activity presumably caused by the presence of adamantyl ring joined to acetophenone~~. Compound **III** (IC_{50} = 205.4 ± 3.56 *u*M) having fluorine at C-23 position of the acetophenone moiety showed slightly less but still remarkable activity as compared to the standard (EDTA). The less activity of this compound may be due the fluorine moiety, which may be produce steric hindrance. Surprisingly compound **V** having uracil moiety and compound **V** having un-substituted benzene in place of acetophenone was found to be completely inactive suggesting that activity in compound **IV** and **III** results from the presence of adamantyl ring as well as from the substitution of flourine on acetophenone ring/ sterically less hindered orientation needed for the interaction of enzyme. Infect this hypothesis is further supported by compound **II** (IC_{50} = 441 ± 10.33 *u*M) that also showed excellent activity more than the standard acarbose (IC_{50} = 906 ± 6.387 *u*M), but less activity than compound **IV** and **III** caused by the change of methoxyl group in position of flouro group.

In phosphodiesterase-I enzyme inhibition assay the compound **III** (IC_{50} = 89.6 ± 1.50 *u*M) showed excellent inhibitory activity caused by the presence of fluorine on acetophenone ring. The compound **II** (IC_{50} = 187.33 ± 3.3) also showed potent activity superior to standard EDTA (IC_{50} = 277.69 ± 1.50) having methoxy group in place of fluorine. The more activity of compound **III** than the compound **II** are due to more electron withdrawing effect of fluorine as compared to having methoxy group. The Compound **VI** (IC_{50} = 361.1 ± 7.23 *u*M), benzene substituted analog exhibited less activity as compared to **III** and **IV** by the change in substitution group at acetophenone moiety.

After analyzing the results obtained by both α-glucosidase and phosphodiesterase-I enzymes inhibition assay, the compound **IV** is the most active compound of the series in α-glucosidase inhibition assay and was completely inactive in phosphodiesterase-I assay.

~~Compound **III,** fluorine analogue is the most active in phosphodiesterase-I assay and second most active structure of the series in the α-glucosidase assay suggesting that the flourine group at position C-23 of the acetophenone ring provides a sterically favored orientation for a fruitful and most significant interaction with enzymes. While compound **V** having uracil substitution instead of fluorine was unlikely to interfere with carbohydrates digestion and glycoprotein and hence found to be completely inactive in both assays.~~

reported structure was determined as (**3)**, as isogospherol.[17]