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Original Research Article

(1H-pyrrolo [2,3-b] pyridine)7-Azaindole Derivatives and their antiurease, Phosphodiesterase and beta-glucuronidase Activity

Abstract: As a part of our program to discover novel analogues of 7-azaindole (1H-pyrrolo [2,3-b] pyridine) having useful biological activities, 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 beta-glucuronidase Inhibitory potential. Compounds **10**, **8, 9** were found to be urease inhibitors with IC_{50} = 255.1 ± 6.62 μM, 133.3 ± 0.46 μM and 2.19 ± 0.37μM respectively. Compound **9** (IC_{50} = 2.19 ± 0.37*u*M) showed potent urease inhibitory potential than standard thiourea (IC_{50} = 21.00 ± 0.01μM). However, compounds **8** (IC_{50} = 133.3 ± 0.46 μM), and **10** (IC_{50} = 255.1 ± 6.62 μM), exhibited activities close to the standard. Compound **2** (IC_{50} = 20.83 ± 0.234*u*M) showed potent phosphodiesterase inhibitory potential than standard EDTA (IC_{50} = 274 ± 0.007*u*M). Besides this, compounds **1-14** were analyzed for beta-glucuronidase inhibition activity. Compound **8** showed good beta-glucuronidase Inhibition activity 192.6 ± 3.53 (1,4,lactone D saccharic acid, IC_{50} = 48.4 ± 1.24 *u*M).

Keywords: *7-Azaindole,* Urease, Beta-glucuronidase Inhibition, synthesis, Phosphodiesterase

Abbreviations: *HMBC, Heteronuclear Multiple Bond Connectivity; PDB, Protein Data Bank; VDCs, voltage dependent Ca21 channels,*

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 [6-9]. Different azaindole compounds were reported for analgesic, blood pressure lowering and anti-inflammatory activities and acting as effective coronary vasodilator, cardiovascular and potent hypotensive agent [10]. Other indole derivatives were prepared as cyclooxygenase inhibitors and were found useful as anti-inflammatory agents [11-12]. These azaindole structures were expected to enhance the cytotoxicity towards tumor cell lines through stronger hydrogen bonding with the target

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enzymes. Saify *et.al.* synthesized a series of 7-azaindole derivatives having important antimicrobial response against gram positive and gram negative bacteria [15-18]. Synthesis and investigation of azaindole derivatives for different pharmacological and biological effects were an important subject of research. Previously a number of novel aza indole compounds has been reported. [13-14, 21]. 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 (H-bond donor and acceptor in 1,3-position) to dock into the adenine binding pocket [20]. However, no work has been reported on the β-glucuronidase, and urease inhibition activity of aza-indole derivatives.

A variety of enzyme inhibition techniques are available for drug discovery and drug designing programs. In our presant course work we designed different derivatives of aza-indole in which we replaced different groups with phenacyl chain at different position and studied β-glucuronidase inhibition as well as other enzyme inhibition including urease and phosphodiesterase.

Enzyme inhibition is an interesting area of research in the field of medicinal chemistry. Its significance is also due to its very easy and *in vitro* method of screening. A variety of enzyme inhibition techniques are available for drug discovery and drug designing programs. A specific inhibitor interacts with a target enzyme in order to prevent its undesirable activities. To develop antiulcer drugs a number of urease inhibitors are currently targeted. A specific inhibitor interacts with a target enzyme in order to prevent its undesirable activities. Activities of Ureases (E.C 3.5.1.5) have been shown to be an important virulence determinant in the pathogenesis of many clinical conditions, which is detrimental for human and animal health as well as for agriculture. 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 is also known to be a major cause of pathologies induced by *Helicobacter pylori* (HP), which allows bacteria to survive at low pH of the stomach during colonization and, therefore, plays an important role in the pathogenesis of gastric and peptic ulcer (including cancer) [23]. In agriculture, high urease activity causes significant environmental and economic problems by releasing abnormally large amounts of ammonia into the atmosphere during urea fertilization. This further induces plant damage primarily by

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depriving them from their essential nutrient and secondly ammonia toxicity, which increase the pH of the soil [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. The enzyme is present in many organs, body fluids, blood cells, liver, spleen, kidney, gastric juice, lung, muscle, bile, urine and serum. In certain disease such as cancer, inflammatory joint disease, some hepatic diseases and AIDS the activity of β-glucuronidase is increased. Human β-glucuronidase also has a role in the deconjugation of glycosaminoglycans. Endogenous biliary β-glucuronidase deconjugates the glucuronides of bilirubin and causes the development of cholelithiasis in human bile. Liver damage cause 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 inhibitos such as 8-hydroxytriceine glucuronide, isovitexin trihydroxy pipecolic acid, scoparic acid A and C have already been isolated from different plants and some are used clinically [26-29].

As a result of our continued interest in 1H pyrrolo (2,3-b) pyridine 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 urease and phosphodiesterase of some new derivatives.

We have carried out a virtual screening of designed compounds with the aim to find novel enzyme inhibitors. The biological activities and biological energies of compound **1-14** were carried out. To find selective inhibitor these compounds were also screened for urease, and phosphodiesterase inhibition. 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.

During the course of synthesis of various derivatives of aza-indole, compound **2** attracted our attention as potential phosphodiesterase inhibitor (IC_{50} = 20.83$\pm$0.234 μM). In the present study, *in vitro* activity of fourteen 7-Azaindole derivatives **2–14** were evaluated. We report in this paper

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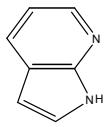
the synthesis and β-glucuronidase inhibition as well as urease and phosphodiesterase inhibitory properties of the 7-aza indole compounds and their mechanism of inhibition.

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 (Scheme-**1**). Reflux reaction mixtures 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 synthesised as a part of study complementary to beta-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 Scheme-**1**.

The ^{1}H-NMR spectra were specially helpful in deciding the substitution pattern at the different positions of phenacyl part of the molecule. The molecular ion peaks of the compounds were obtained from EIMS spectra [34]. A series of 7-azaindole derivatives were prepared by quaternization with phenethyl bromide and substituted phenacyl halides, the side chain thus possessing certain features common to those present in sympathomimetic amines and related compounds.



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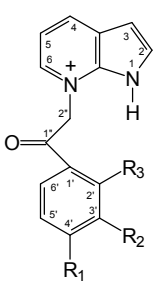
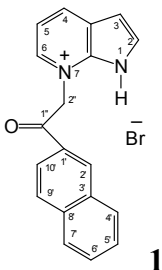


	R1	R2	R3
2	OH	OH	H
3	H	NO_2	H
4	H	H	NO_2
5	NO_2	H	H
6	F	H	F
7	F	H	H
8	Cl	H	H
9	OCH_3	H	H



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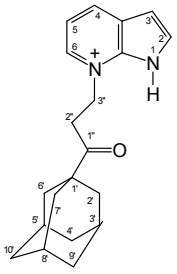

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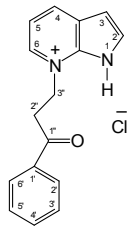

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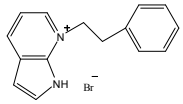

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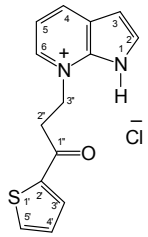

14)

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**, compound **2**, **8**, **9**, **10**, **11**, **13** and **14** showed reasonable % inhibition, while compounds **3**, **6**, **7** and **12** displayed no activity. The Compound **8** was selected for IC_{50} values and this compound showed IC_{50} value 192.6 ±3.53 μM. Compound **8** containing chloro derivative displayed maximum inhibition (IC_{50} 192.6 ±3.53 μM), while the compound **14** showed 47.1 % inhibition was the second potent inhibitor of *β*-glucuronidase. Compound **10** and **13** (naphthyl and benzyl derivatives) showed almost same level of inhibition with 20 and 37%.

Due to wide range biological importance of 7-azaindole, here we reported the potential of 7-azaindole against urease. The urease is good target for the gastric and peptic ulcer [35]. 7-azaindole derivatives **1-14** were screened against urease enzyme according to literature protocol [36,37]. 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 ± 0.11 μM) (Table-1). Compound **1-14** were screened for urease inhibitory activity (Table **1)**. When parent compound **1** was screened along with its newly synthesized derivatives **2-14**, it was found that parent

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compound showed weak enzyme inhibitory activity (2.9%) at 0.5 *m*M. Among the synthesized analogues, compound **13** exhibited weak activity (6.7%) against urease enzyme. Compound **9** exhibited activity comparable with standard, while compound **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 compound **8**, **9**, **10** (chloride, methoxy derivative). These compounds were selected for IC_{50} value and these showed 133.3±0.46, 2.19±0.37 and 255.1±6.62 μM IC_{50} values. Compound **9** showed remarkable inhibition with an IC_{50} value of 2.19± 0.37, 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 are also more potent, very close to the standard drug.

As we can see that attaching the different molecules at N atom of aza-indole making 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 halides analogues compound **8** (chloro derivative) showed better effect as compared to compound **6** (flouro derivative).

Biological Activity

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

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 most active. Compounds **3** and **5** showed week activity. Compound **2** showed remarkable inhibition with an IC_{50} value of 20.83$\pm$0.234μM, which is about twenty times more active than the standard drug EDTA (IC_{50} = 274 $\pm$ 0.007 μM). The result indicates that OH substitution in phenacyl ring at 3,4

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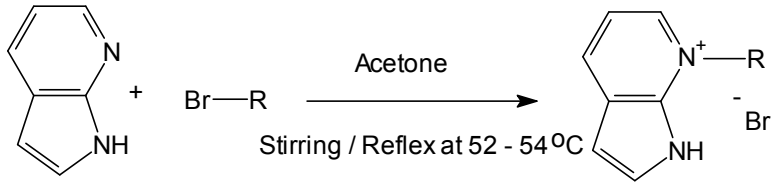
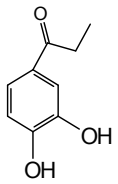
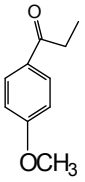
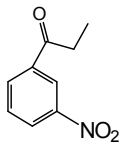
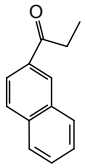
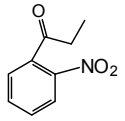
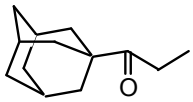
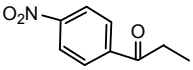
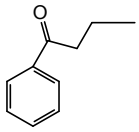
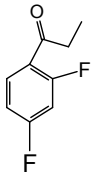
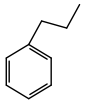
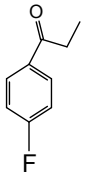
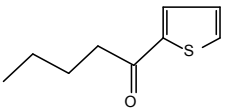
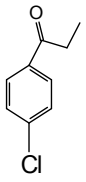
175 position (meta, para for compound **2**) is making significant change in the activity with IC_{50} value
176 20.83 $\pm$0.234μM. Results of phosphodiesterase activity are represented in Table **1.**



177
178 **1** **2a-14a** **2-14**

Reactants

No.	**R**	**No.**	**R**
2a		9a	
3a		10a	
4a		11a	
5a		12a	
6a		13a	
7a		14a	
8a			

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Scheme-1: Synthesis of 7-azaindole derivatives **1-14**

investigation for beta-glucuronidase inhibition activity opposite results have been observed as compared to urease inhibition. Approximately similar inhibition of beta-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 significance difference in the activity.

Table – I: Beta- glucuronidase, Phosphodiesterase and Urease Inhibition activity of 7- azaindol derivatives:

S.No.	**Beta- glucuronidase Inhibition**			**Phosphodiesterase Inhibition**			**Urease Inhibition**		
	Conc.(mM)	% INHIBITION	IC_{50} ±SEM (μM)	Conc. (*m*M)	% INHIBITION	IC_{50} ±SEM (*μ*M)	Conc. (*m*M)	% INHIBITION	IC_{50} ±SEM (*μ*M)
1	-	-	-	0.4	17.9	-	0.5	2.9	-
2	0.5	6.3	-	1	86.0	20.83±0.234	0.5	44.4	-
3	0.5	-VE	-	1	5.6	-	ND	-	-
4	0.5	-VE	-	0.2	18.9	-	ND	-	-
5	0.5	-VE	-	1	6.7	-	ND	-	-
6	0.5	-VE	-	1	-VE	-	0.5	41.8	-
7	0.5	-VE	-	1	17.1	-	ND	-	-
8	0.5	78.5	192.6 ± 3.53	1	-VE	-	0.5	74.8	133.3+_0.46
9	0.5	13.6	-	1	VE	-	0.5	75.8	2.19± 0.37
10	0.2	20.0	-	0.2	20.6	-	0.5	66.6	255.1±6.62
11	0.5	13.0	-	0.2	17.8	-	0.5	16.10	-
12	0.5	-VE	-	1	-VE	-	0.5	21.8	-
13	0.5	36.8	-	1	-VE	-	0.5	6.7	-
14	0.5	47.1	-	1	-VE	-	0.5	30.9	-
	1,4,lactone D saccharic acid		48.4±1.24	EDTA		274 ± 0.007	Thiourea		21± 0.11

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Materials and methods

Experimental

General procedure

Reagents were procured from Aldrich Chemical Company. The entire solvents were reagents grade. Reactions were monitored by TLC using pre-coated silica gel, GF-254 and Analytical thin layer chromatography (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 MHz.

General method of preparation of compounds

To a stirred equimolar, quantity of 7-azaindole in acetone (20-25 ml) 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^{o}C. The process of reaction is 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.

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226 **β-Glucuronidase Enzyme Inhibition**

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228 β-Glucuronidase (E.C. 3.2.1.31) from *E. coli*, (*p*-nitrophenyl-β-D-glucuronide) was obtained
229 from Sigma Chemical Co. Other reagents were also purchased from commercial sources. β-
230 Glucuronidase activity was determined by measuring the absorbance at 405 nm of *p*-nitrophenol
231 formed from the substrate by the spectrophotometric method. The total reaction volume is 250
232 μl. The reaction mixture contained 185 μl of 0.1 M acetate buffer, 5μl of test compound solution,
233 10μl of enzyme solution incubated at 37 °C for 30 min. The plates were read on a multiplate
234 reader (SpectraMax plus 384) at 405 nm after the addition of 50 μl of 0.4*m*M *p*-nitrophenyl-β-D-
235 glucuronide. All assays were run in triplicate [31]. The IC_{50} values were calculated using the EZ-
236 Fit Enzyme Kinetics program, Perrella Scientific Inc., Amherst, U.S.A.

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238 **Urease Enzyme Inhibition**

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240 Reaction mixtures comprising 1 unit of urease enzyme (*Bacillus pasteurii or Jack bean*) solution
241 and 55μl of buffers containing 100*m*M urea were incubated with 5μl of test compounds (1*m*M
242 concentration) at 30°C for 15 min in 96-well plates.
243 Urease activity was determined by measuring ammonia production using the indophenol method
244 as described by Weatherburn [32]. Briefly, 45μl each of phenol reagent (1% w/v phenol and
245 0.005% w/v sodium nitroprusside) and 70μl of alkali reagent (0.5% w/v NaOH and 0.1% active
246 chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured
247 after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed
248 in triplicate in a final volume of 200μl. The results (change in absorbance per min) were
249 processed by using Soft- Max Pro software (Molecular Device, USA). All reactions were
250 performed in triplicate. All the assays were performed at pH 8.2 (0.01 M $K_2HPO_4.3H_2O$, 1*m*M
251 EDTA and 0.01 M $LiCl_2$). Percentage inhibitions were calculated from the formula 100-
252 $(OD_{testwell}/OD_{control})$ x 100. Thiourea was used as the standard inhibitor of urease.

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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 [33] 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^{o}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, Melting point = 105-107 ^{o}C.

1-[3-(3,4-dihydroxyphenyl)3-oxoethyl]-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, Melting Point = 224 + 2^{o}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,

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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: = $C_{15}H_{12}N_3O_3Br$, EI MS: *m/z* = 362.178 amu, Melting Point = 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: $C_{15}H_{12}N_3O_3Br$, EI MS: *m/z* = 362.18 amu, Melting Point = 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).

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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, Melting Point = 267 ± 2^{o}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").

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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, Melting Point = 287 + 2^0C, UV λ_{max} (MeOH) nm = 299.6, 226.0, IR ν_{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

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(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').

Declaration of interest

The authors report no conflicts of interest.

Conclusion

Over all result are significant and encouraging and because of their phosphodiesterase, urease and *β*-glucuronidase inhibition potential they can be selected as a useful lead molecules for designing effective therapeutic agent to treat these diseases. 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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