Research Article

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Future Medicinal Chemistry

Synthesis and drug efficacy validations of racemic-substituted benzimidazoles as antiulcer/antigastric secretion agents

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Aim: Due to their effective binding affinity to receptors which are responsible for various diseases, benzimidazoles are often bioactive. Present study intended and carried out to synthesis, characterize and develop benzimidazole-based antiulcer drugs. **Materials & methods:** Established **8a–l** were evaluated for gastric antisecretory/antiulcer properties using freshly prepared H^+-K^+-ATPase from goat fundus mucosa. Molecular docking was carried out to unveil best binding affinities with H^+-K^+-ATPase (protein data bank ID: 2XZB). **Results:** The obtained least inhibitory constant of **8a–l** (18–92 nM) was comparable to the *in vitro* H^+-K^+-ATPase inhibition (IC_{50}: 24–122 nM). Furthermore, the lethal effect of **8a–l** to colon cancerous cells and nonharm effect to the normal cells was recognized through cytotoxicity studies. **Conclusion:** After all *in silico, in vitro* experimental and structure–activity relationship predictions, the antiulcer druggability potential of **8a–l** was recognized. A future drug development study for the most potent compounds among **8a–l** is strongly indorsed.

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Keywords: antiulcer • benzimidazole • cytotoxicity • H^+-K^+-ATPase • selective oxidation

In synthetic organic chemistry, sulfoxide-substituted benzimidazoles and their associated derivatives are playing a significant role in novel drug development researches through their valuable medicinal effects against numerous disease targets (e.g., omeprazole) [1]. In the chemical synthesis, these benzimidazole-sulfoxide-based compounds are perpetually set from the interrelated sulfides, where halogen [2,3] and metals-mediated oxidizing systems are usually involved [4]. Astonishingly, only a few reports are available to explain the controlled synthesis of sulfoxides using various oxidants [5]. In fact, the sulfide oxidation has several disadvantages including the long reaction time, problematic reaction condition, the need of expensive oxidants, undesired side reactions to other functional groups and less yield [2–4]. To overcome this, recently, zirconium chloride and hydrogen peroxide are used for the selective oxidation of sulfides to sulfoxides, but at the same time, excess use of oxidant in the synthesis of sulfoxides is commonly considered as a drawback [6]. According to a previous report, sulfide (30%)/H_2O_2/$ZrCl_4$, 2:14:4 in methanol and MoO_2Cl_2 was used as an efficient catalyst for several organic transformations [8]. Apart from this, oxidants such as $VO(acac)_2$/H_2O_2 [7], $MoO_2(acac)_2$/H_2O_2 [8], NBS/β-cyclodextrin [9], oxone [10] and chromium reagents [11], are used efficiently for sulfide oxidation as well as oxidative cleavage of oximes [12]. Considering all the above points, in this study, we have used $(NH_4)_6Mo_7O_{24}.4H_2O$/H_2O_2 as an oxidant for the efficient and selective oxidation of sulfides to sulfoxides [sulfide/50% H_2O_2/$(NH4)_6Mo_7O_{24}.4H_2O$, 1:1.05:0.015 in methanol water].

Based on the structural and functional analyses, the bioactivity potency of the present study compounds was predetermined as ulcer medications. H^+-K^+-ATPase enzyme inhibition study was proposed to be evaluated. Because, H^+-K^+-ATPase induces the unnecessary gastric secretion which leads in to ulcer. While discussing the bioactivity prospects from decade to decade, benzimidazoles are showed excellent medicinal values and they have been established well as a broad-spectrum small molecules that are including, but not only limited to lipase inhibitors [13], antifungal [14], antimicrobial [15], antioxidant [16], anti-inflammatory [17], anticancer [18],

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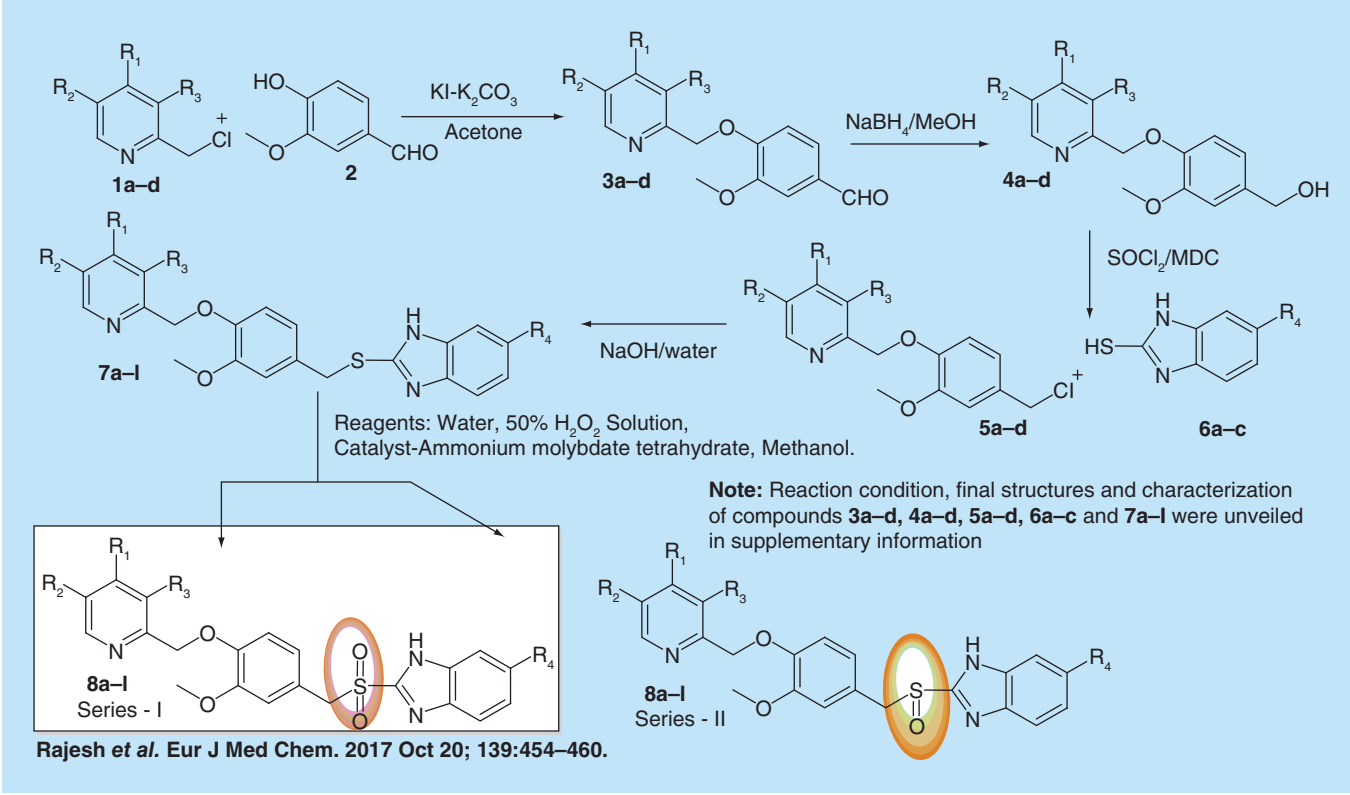

Figure 1. Route of synthesis and reaction conditions of 8a–l.

antihypertensive [19], antiviral [20], antihistamine [21], analgesic [22], anticonvulsant [23], antiulcer [24]. Omeprazole, lansoprazole and pantoprazole are the well-known benzimidazole-based proton pump inhibitors (PPIs; Figure 1). PPIs, the most potent defenders against gastric acid secretions [25], are also used in stress gastritis and gastric ulcer prevention in acute care [26]. In addition, PPIs are also involved in anti-*Helicobacter pylori* treatment [27]. Most prominently, PPIs have been reported to have significant medicinal values against gastroesophageal reflux disease including symptomatic endoscopy-negative reflux disease [28,29]. In this study, **8a–l** were synthesized with necessary modifications in continuation of our previous report [30] by maintaining benzimidazole as the backbone and removing a carboxyl group from the previous form to evaluate H^+-K^+-ATPase inhibition properties for its use in antiulcer therapeutics.

Materials & methods

Chemistry

All solvents and chemicals were obtained from Sigma-Aldrich. The reaction progress was monitored by thin-layer chromatography using precoated SiO_2 gel (HF_{254} 200 mesh) aluminum plates (E Merck). The melting points of synthesized compounds were analyzed in Buche apparatus with one-end-opened capillary tube. Specific optical rotation (SOR) of these compounds was recorded on the AUTOPOL-V analyzer. The infra-red (IR) spectra of these compounds were recorded on ABB Bomen Fourier-Transform Infra-Red (FTIR) spectrometer MB 104 with KBr Pellets. ^{1}H NMR and spectra were recorded on 400 MHz-Broker DPX 200 and Variant 300 MHz spectrometers by using tetramethylsilane as an internal standard. Chemical shifts were reported as in parts per million downfield from tetramethylsilane. Spin multiplicities were described as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), p (pentet) and m (multiplet). Coupling constants were reported in Hertz. Mass spectra were recorded on a Finnigan MAT 8230 mass spectrometer at 200°C, 70 eV with trap current of 200 IA and 4 kV acceleration voltages.

General procedure for preparation of 8a–l

Earlier, we have synthesized, characterized and reported the substituted 4-[2-pyridylmethoxy] phenyl methylthio-substituted benzimidazole (**7a–l**) [30]. To achieve the substituted benzimidazoles (**8a–l**), 1.0 gm of **7a–l** was charged into 15 ml of methanol and to this 0.000016 mol of ammonium molybdate tetrahydrate and 0.0088 mol of hydrogen peroxide were slowly added. The reaction mass was stirred for about 2–3 h at 27 ± 3°C. After the thin-layer chromatography complies, the reaction mass was quenched with 1% sodium thiosulfate solution and material was extracted with Methylene dichloride (MDC). The organic layer was concentrated under vacuum at 35°C. The product was then isolated with methanol and *n*-Hexane, and was dried under vacuum to obtain **8a–l**.

Racemic-2-(4-((4-(2,2,2-trifluoroethoxy)-3-methylpyridin-2-yl)methoxy)-3-methoxy benzylsulfinyl)-1H-benzo[d] imidazole (8a)

White color solid with 92% yield; $C_{24}H_{22}F_3N_3O_4S$: Melting range (MR) 205.8–207.6°C, IR (KBr, γ_{max} in cm^{-1}): 3431.2 (N-H), 3059.8 (C-H, aromatic ring), 2887.9 (C-H, methoxy), 1586.8 (C=N, aromatic ring), 1511.7 (C=C, aromatic ring), 1141.9 (C-N, imidazole), 1141.9 (C-O-C), 1041.0 (C-F); ^{1}H NMR (DMSO-d6) δ: 2.173 (s, 3H), 3.328 (s, 4H), 4.358–4.401 (d, 1H), 4.575–4.617 (d, 1H), 4.858–4.946 (q, 2H), 5.093 (s, 2H), 6.505 (s, 1H), 6.702–6.724 (d, 1H), 6.983–7.012 (d, 1H), 7.122–7.142 (d, 1H), 7.245–7.276 (dd, 2H), 7.603–7.633 (dd, 2H), 8.312–8.332 (d, 1H); ^{13}C NMR (DMSO-d6) δ: 164.3, 154.5, 150.0, 149.0, 147.7, 123.8, 123.0, 120.3, 114.5, 113.9, 107.0, 71.6, 69.3, 65.5, 61.8, 59.2, 56.2, 55.8, 10.81; ^{19}F NMR (DMSO-d6) δ: -72.752, -72.720, -72.692; MS *m/z* 506.1 $[M+H]^+$; High-Resolution Mass Spectrometry (HRMS) for $C_{24}H_{22}F_3N_3O_4S$, Calculated $[M^+]$ *m/z* 505.5122; Found 505.5128.

Racemic-2-(4-((4-(2,2,2-trifluoroethoxy)-3-methylpyridin-2-yl)methoxy)-3-methoxy benzylsulfinyl)-5-methoxy-1H-benzo[d]imidazole (8b)

Off-white color solid with 87% yield; $C_{25}H_{24}F_3N_3O_5S$: MR 165.2–166.4°C, IR (KBr, γ_{max} in cm^{-1}): 3434 (N-H), 3118.9 (C-H, Ar), 2955.9 (C-H, methoxy), 1630.9 (C=N, imidazole), 1585.1 (C=N, aromatic ring), 1515.7 (C=C, aromatic ring), 1167.6 (C-N, imidazole), 1136.1 (C-O-C), 1041.4 (C-F), 741.4 (C-S); ^{1}H NMR (DMSO-d6) δ: 2.177 (s, 3H), 3.372 (s, 3H), 3.798 (s, 3H), 4.355–4.399 (d, 1H), 4.557–4.599 (d, 1H), 4.863–4.948 (q, 2H), 5.100 (s, 2H), 6.529 (s, 1H), 6.696–6.720 (d, 1H), 6.902–7.016 (m, 3H), 7.128–7.148 (d, 1H), 7.407 (s, 1H), 8.318–8.336 (d, 1H), 13.188 (s, 1H); ^{13}C NMR (DMSO-d6) δ: 164.2, 154.5, 150.0, 149.0, 147.7, 107.0, 71.6, 69.3, 65.5, 61.7, 59.2, 56.2, 55.7, 10.8; ^{19}F NMR (DMSO-d6) δ: -72.755, -72.724, -72.692; MS *m/z* 536.0 $[M+H]^+$; HRMS for $C_{25}H_{24}F_3N_3O_5S$, Calculated $[M^+]$ *m/z* 535.5382; Found 535.5383.

Racemic-2-(4-((4-(2,2,2-trifluoroethoxy)-3-methylpyridin-2-yl)methoxy)-3-methoxy benzylsulfinyl)-5-(difluoromethoxy)-1H-benzo[d]imidazole (8c)

Off-white color solid with 85% yield; $C_{25}H_{22}F_5N_3O_5S$: MR 185.0–186.2°C, IR (KBr, γ_{max} in cm^{-1}): 3430.0 (N-H), 3151.9 (C-H, Ar), 1625.1 (C=N, imidazole), 1584.6 (C=N, aromatic ring), 1515.8 (C=C, Ar), 1166.9 (C-N, imidazole), 1135.2 (C-O-C), 1040.5 (C-F), 812.3 (C-S); ^{1}H NMR (DMSO-d6) δ: 2.175 (s, 3H), 3.356 (s, 4H), 4.349–4.393 (d, 1H), 4.573–4.617 (d, 1H), 4.858–4.946 (q, 2H), 5.095 (s, 2H), 6.509–6.514 (dd, 1H), 6.959–7.014 (d, 1H), 6.959, 7.207 and 7.456 (t, 1H, due to C-F coupling), 7.093–7.142 (dd, 2H), 7.401–7.405 (d, 1H), 7.627–7.655 (d, 1H), 8.312–8.332 (d, 1H), 13.2 (bs, 1H); ^{13}C NMR (DMSO-d6) δ: 167.5, 164.3, 154.5, 150.0, 147.7, 123.8, 123.0, 120.3, 114.5, 113.9, 107.0, 71.6, 69.3, 65.5, 61.8, 59.2, 56.2, 55.7, 10.8; ^{19}F NMR (DMSO-d6) δ: -81.552, -81.288, -72.759, -72.727, -72.696; MS *m/z* 572.1 $[M+H]^+$; HRMS for $C_{25}H_{22}F_5N_3O_5S$, Calculated $[M^+]$ *m/z* 571.5190; Found 571.5190.

Racemic-2-(4-((4-methoxy-3,5-dimethylpyridin-2-yl)methoxy)-3-methoxybenzyl sulfinyl)-1H-benzo [d]imidazole (8d)

White color solid with 89% yield; $C_{24}H_{25}N_3O_4S$: MR 208.8–210.2°C, IR (KBr, γ_{max} in cm^{-1}): 3420.4 (N-H), 3066.0 (C-H, Ar), 2935.2 (C-H, methoxy), 1589.1 (C=N, imidazole), 1630.9 (C=N, aromatic ring), 1516.0 (C=C, Ar), 1155.6 (C-N, imidazole), 1108.6 (C-O-C), 1003.9 (C-F), 733.2 (C-S); ^{1}H NMR (DMSO-d6) δ: 2.265 (s, 6H), 3.477 (s, 3H), 3.743 (s, 3H), 4.373–4.417 (d, 1H), 4.586–4.630 (d, 1H), 5.060 (s, 2H), 6.494–6.498 (d, 1H), 6.700–6.733 (dd, 1H), 7.003–7.030 (d, 1H), 7.278–7.308 (dd, 2H), 7.629 (broad s, 2H), 8.209 (s, 1H), 13.354 (s, 1H); ^{13}C NMR (DMSO-d6) δ: 165.0, 154.5, 150.0, 149.0, 147.7, 139.2, 135.0, 131.9, 123.8, 120.3, 114.5, 113.9, 107.0, 61.8, 59.2, 56.2, 15.8, 10.8; MS *m/z* 452.1 $[M+H]^+$; HRMS for $C_{24}H_{25}N_3O_4S$, Calculated $[M^+]$ *m/z* 451.5410; Found 451.5412.

Racemic-2-(4-((4-methoxy-3,5-dimethylpyridin-2-yl)methoxy)-3-methoxybenzyl sulfinyl)-5-methoxy-1H-benzo[d]imidazole (8e)

Off-white color solid with 79% yield; $C_{25}H_{27}N_3O_5S$: MR 179.8–181.6°C, IR (KBr, γ_{max} in cm^{-1}): 3401.0 (N-H), 3081.8 (C-H, Ar), 2833.1 (C-H, methoxy), 1622.0 (C=N, imidazole), 1591.1 (C=N, Ar), 1517.1 (C=C, aromatic ring), 1151.2 (C-N, imidazole), 1105.8 (C-O-C), 774.1 (C-S); ^{1}H NMR (DMSO-d6) δ: 2.223 (s, 6H), 3.332 (s, 3H), 3.732 (s, 3H), 3.795 (s, 3H), 4.351–4.393 (s, 1H), 4.555–4.599 (s, 1H), 5.054 (s, 2H), 6.536–6.542 (d, 1H), 6.700–6.733 (dd, 1H), 6.876–6.913 (d, 1H), 7.001–7.027 (d, 1H), 7.063–7.069 (d, 1H), 7.506–7.537 (d, 1H), 8.195 (s, 1H), -13.0 (bs, 1H); ^{13}C NMR (DMSO-d6) δ: 164.7, 154.9, 150.4, 149.4, 148.1, 139.4, 134.3, 124.2, 123.5, 120.7, 114.9, 114.3, 107.4, 62.2, 59.6, 56.6, 56.13, 15.7, 11.2; MS *m/z* 482.2 $[M+H]^+$; HRMS for $C_{25}H_{27}N_3O_5S$, Calculated $[M^+]$ *m/z* 481.5670; Found 481.5678.

Racemic-2-(4-((4-methoxy-3,5-dimethylpyridin-2-yl)methoxy)-3-methoxybenzylsulfinyl)-5-(difluoromethoxy)-1H-benzo[d]imidazole
(8f)

Off-white solid with 72% yield; $C_{25}H_{25}F_2N_3O_5S$: MR 188.8–1189.2°C, IR (KBr, γ_{max} in cm^{-1}): 3412.0 (N-H), 3072.6 (C-H, Ar), 2940.4 (C-H, methoxy), 1632.5 (C=N, imidazole), 1590.0 (C=N, Ar), 1516.2 (C=C, Ar), 1159.3 (C-N, imidazole), 1132.8 (C-O-C), 1040.4 (C-F), 765.6 (C-S); ^{1}H NMR (DMSO-d6) δ: 2.215 and 2.239 (s, 6H), 3.670 (s, 3H), 3.732 (s, 3H), 4.456 (s, 2H), 5.060 (s, 2H), 6.471 (s, 1H), 6.823, 7.095 and 7.383 (t, 1H, due to C-F coupling), 6.830–6.858 (dd, 1H), 6.911–6.944 (dd, 1H), 6.999–7.027 (d, 1H), 7.080–7.087 (d, 1H), 7.181–7.188 (d, 1H), 7.346–7.355 (d, 1H), 8.195 (s, 1H); ^{13}C NMR (DMSO-d6) δ: 165.0, 154.7, 150.0, 149.0, 147.6, 139.3, 135.1, 131.8, 123.7, 123.6, 120.3, 114.5, 113.9, 107.0, 71.5, 61.8, 59.2, 56.2, 15.7, 11.2; ^{19}F NMR (DMSO-d6) δ: -80.565, -80.298, MS *m/z*, 518.0 $[M+H]^+$; HRMS for $C_{25}H_{25}F_2N_3O_5S$, Calculated $[M^+]$ *m/z* 517.5478; Found 517.5482.

Racemic-2-(4-((3,4-dimethoxypyridin-2-yl)methoxy)-3-methoxybenzylsulfinyl)-1H-benzo[d] imidazole (8g)

Off-white color solid with 45% yield; $C_{23}H_{23}N_3O_5S$: MR 182.4–183.6°C, IR (KBr, γ_{max} in cm^{-1}): 3442.1 (N-H), 3069.2 (C-H, Ar), 2943.1 (C-H, methoxy), 1585.3 (C=N, Ar), 1514.2 (C=C, Ar), 1266.5 (C-N, imidazole), 1149.1 (C-O-C), 749.5 (C-S); ^{1}H NMR (DMSO-d6) δ: 3.647 (s, 3H), 3.746 (s, 3H), 3.891 (s, 3H), 4.492 (s, 2H), 5.010 (s, 2H), 6.923–7.448 (m, 6H), 8.192 (s, 2H), 8.208 (s, 1H), 12.519 (s, 1H); ^{13}C NMR (DMSO-d6) δ: 159.3, 149.6, 149.5, 148.9, 146.9, 145.6, 144.8, 123.5, 119.6, 113.9, 113.4, 108.0, 67.4, 61.6, 61.4, 56.0, 55.2; MS *m/z* 454.2 $[M+H]^+$; HRMS for $C_{23}H_{23}N_3O_5S$, Calculated $[M^+]$ *m/z* 453.5130; Found 453.5134.

Racemic-2-(4-((3,4-dimethoxypyridin-2-yl)methoxy)-3-methoxybenzylsulfinyl)-5 methoxy-1H -benzo[d] imidazole (8h)

White color solid with 72% yield; $C_{24}H_{25}N_3O_6S$: MR 171.8–173.2°C, IR (KBr, γ_{max} in cm^{-1}): 3426.1 (N-H), 304.3 (C-H, Ar), 2872.5 (C-H, methoxy), 1626.4 (C=N, imidazole), 1587.0 (C=N, Ar), 1513.9 (C=C, Ar), 1136.2 (C-N, imidazole), 1114.2 (C-O-C), 734.1 (C-S); ^{1}H NMR (DMSO-d6) δ: 3.640 (s, 3H), 3.746, 3.763 ($_2$s, 6H), 3.893 (s, 3H), 4.448 (s, 2H), 5.008 (s, 2H), 6.730–6.769 (dd, 1H), 6.910–7.030 (m, 4H), 7.120–7.40 (d, 1H), 7.325–7.353 (d, 1H), 8.192–8.209 (d, 1H), 12.368 (s, 1H); ^{13}C NMR (DMSO-d6) δ: 159.19, 149.5, 149.5, 148.9, 145.7, 144.8, 123.5, 122.1, 119.8, 113.7, 113.4, 108.0, 94.4, 67.5, 61.6, 61.4, 55.9, 55.9, 55.3; MS *m/z* 484.2 $[M+H]^+$; HRMS for $C_{24}H_{25}N_3O_6S$, Calculated $[M^+]$ *m/z* 483.5390; Found 483.5393.

Racemic-2-(4-((3,4-dimethoxypyridin-2-yl)methoxy)-3-methoxybenzylsulfinyl)-5-(difluoromethoxy)-1H-benzo[d]imidazole
(8i)

White color solid with 47% yield; $C_{24}H_{23}F_2N_3O_6S$: MR 193.8–194.6°C, IR (KBr, γ_{max} in cm^{-1}): 3440.8 (N-H), 3031.8 (C-H, Ar), 2863.7 (C-H, methoxy), 1625.2 (C=N, imidazole), 1588.2 (C=N, aromatic ring), 1514.0 (C=C, Ar), 1165.0 (C-N, imidazole), 1127.2 (C-O-C), 1034.2 (C-F), 718.2 (C-S); ^{1}H NMR (DMSO-d6) δ: 3.653 (s, 3H), 3.748, 3.893 (s, 3H), 4.475 (s, 2H), 5.010 (s, 2H), 6.879–7.140 (m, 6H), 6.879, 7.377 and 7.140 (t, 1H, due to C-F coupling), 7.724–7.230 (d, 1H), 7.401–7.430 (d, 1H), 8.192–8.212 (d, 1H); ^{13}C NMR (DMSO-d6) δ: 167.4, 159.3, 149.6, 149.5, 149.0, 146.9, 145.6, 144.8, 123.5, 119.6, 113.9, 113.5, 108.0, 67.4, 61.6, 61.4, 56.0, 55.2; MS *m/z* 520.2 $[M+H]^+$; HRMS for $C_{24}H_{23}F_2N_3O_6S$, Calculated $[M^+]$ *m/z* 519.5198; Found 517.5200.

Racemic-2-(4-((4-(3-methoxypropoxy)-3-methylpyridin-2-yl)methoxy)-3-methoxybenzyl sulfinyl)-1H-benzo [d]imidazole (8j)

White color solid with 74% yield; $C_{26}H_{29}N_3O_5S$: MR 181.4–182.6°C, IR (KBr, γ_{max} in cm^{-1}): 3432.8 (N-H), 3054.6 (C-H, Ar), 2884.3 (C-H, methoxy), 1590.7 (C=N, Ar), 1513.0 (C=C, Ar), 1191.1 (C-N, imidazole), 1161.7 (C-O-C), 744.9 (C-S); ^{1}H NMR (DMSO-d6) δ: 2.092–2.076 (d, J = 6.4, 3H), 2.217 (s, 3H), 3.240 (s, 3H), 3.350 (s, 3H), 3.572–3.541 (t, J = 12.4, 2H), 4.111–4.080 (t, J = 12.4, 2H), 4.595 (s, 2H), 5.075 (s, 2H), 6.353–6.349 (d, J = 1.6, 1H), 6.604–6.580 (m, 1H), 7.376–7.353 (t, J = 9.2, 3H), 8.201–8.187 (d, J = 5.6, 1H), 12.079 (s, 1H); ^{13}C NMR (DMSO-d6) δ: 164.0, 154.3, 149.7, 148.7, 147.4, 146.9, 123.5, 122.7, 119.7, 114.3, 113.5, 106.6, 71.3, 68.9, 65.2, 61.4, 58.9, 55.4, 29.4, 10.4; MS *m/z* 496.2 $[M+H]^+$; HRMS for $C_{26}H_{29}N_3O_5S$, Calculated $[M^+]$ *m/z* 495.5940; Found 495.5940.

Racemic-2-(4-((4-(3-methoxypropoxy)-3-methylpyridin-2-yl)methoxy)-3-methoxybenzyl sulfinyl)-5-methoxy-1H-benzo[d]imidazole (8k)

Off-white color solid with 68% yield; $C_{27}H_{31}N_3O_6S$: MR 143.6–144.4°C, IR (KBr, γ_{max} in cm^{-1}): 3401.9 (N-H), 3093.0 (C-H, aromatic ring), 2932.3 (C-H, methoxy), 1627.2 (C=N, imidazole), 1584.7 (C=N, aromatic ring), 1515.0 (C=C, aromatic ring), 1148.7 (C-N, imidazole), 1126.3 (C-O-C), 745.9 (C-S); ^{1}H NMR (DMSO-d6) δ: 2.207 (s, 2H), 2.268 (s, 3H), 3.268 (s, 3H), 3.349 (s, 3H), 3.554–3.539 (d, J = 6, 2H), 3.796–3.569 (m, 3H), 4.107–4.076 (t, J = 12.4, 2H), 4.568 (s, 2H), 5.057 (s, 2H), 6.361–6.357 (d J = 1.6, 1H), 6.611–6.591 (d, J = 8, 1H), 6.770–6.720 (m, 2H), 7.001–6.973 (m, 1H), 8.191–8.177 (d, J = 8, 1H), 12.048 (s, 1H); ^{13}C NMR (DMSO-d6) δ: 164.0, 154.2, 149.7, 148.7, 147.4, 123.5, 122.8, 120.0, 114.1, 113.6, 106.7, 71.2, 69.0, 65.2, 61.5, 58.9, 55.9, 55.4, 29.4, 10.45; MS *m/z* 526.0 $[M+H]^+$; HRMS for $C_{27}H_{31}N_3O_6S$, Calculated $[M^+]$ *m/z* 525.6200; Found 525.6204.

Racemic-2-(4-((4-(3-methoxypropoxy)-3-methylpyridin-2-yl)methoxy)-3-methoxybenzyl sulfinyl)-5-(difluoro methoxy)-1H-benzo[d]imidazole (8l)

Off-white color solid with 71% yield; $C_{27}H_{29}F_2N_3O_6S$: MR 149.8–151.4°C, IR (KBr, γ_{max} in cm^{-1}): 3426.4 (N-H), 3121.5 (C-H, Ar), 2833.1 (C-H, methoxy), 1626.9 (C=N, imidazole), 1586.1 (C=N, Ar), 1514.5 (C=C, Ar), 1166.2 (C-N, imidazole), 1127.0 (C-O-C), 1038.5 (C-F), 773.7 (C-S); ^{1}H NMR (DMSO-d6) δ: 2.094–2.079 (d, J = 6, 3H), 2.209 (s, 3H), 3.247 (s, 3H), 3.351 (s, 3H), 3.572–3.542 (t, J = 12, 2H), 4.116–4.085 (t, J = 12.4, 2H), 4.576 (s, 2H), 5.039 (s, 2H), 6.382–6.340 (d, J = 16.8, 1H), 6.549–6.524 (d, J = 10, 1H), 6.749–6.675 (m, 2H), 7.165–7.138 (m, 1H), 7.265 (s, 1H), 7.368 (s, 1H), 7.643–7.622 (t, J = 9.2, 3H), 8.157–8.142 (d, J = 6, 1H), 12.793 (s, 1H); ^{13}C NMR (DMSO-d6) δ: 164.0, 154.0, 149.7, 148.7, 148.4, 147.1, 123.4, 122.7, 119.8, 116.1, 114.1, 113.6, 106.7, 70.8, 68.9, 65.3, 61.3, 58.9, 55.4, 29.4, 10.45; MS *m/z* 562.0 $[M+H]^+$; HRMS for $C_{27}H_{29}F_2N_3O_6S$, Calculated $[M^+]$ *m/z* 561.6008; Found 561.6012.

Determination H^+-K^+-ATPase inhibition: preparation enzyme & substrate

Estimating the amount of inorganic phosphate released from ATP is one of the effective ways to determine a drug candidate's H^+-K^+-ATPase inhibitory potential and antiulcer therapeutic values. H^+-K^+-ATPase enzyme was prepared from fresh goat stomach. The inner layer of the stomach was scraped out for parietal cells of mucosa at the gastric fundus [31]. Cells were homogenized using 16-mM Tris buffer (pH 7.4) having 10% Triton X100 and centrifuged at 8000 *g* for 10 min. The obtained supernatant containing H^+-K^+-ATPase enzyme was used to determine inhibition potentials of **8a–l**. The determination of protein content of the crude enzyme was estimated using the Bradford's method using bovine serum albumin as the standard [32]. Different ratio of the reaction mixture at different concentrations was preincubated for 30–90 min at room temperature.

H^+-K^+-ATPase inhibition assay

To determine the amount of inorganic phosphate released from ATP, H^+-K^+-ATPase inhibition assay was carried out. Initially, to the substrate, 2 mM ATP (200 µl), 2 mM MgCl (200 µl) and 10 mM KCl (200 µl) were added and incubated at 37°C for 30 min. Then the assay mixture (4.5% ammonium molybdate and 60% per chloric acid) was added and incubated for 10 min, and then centrifuged at 3000 rpm for 5 min. With 0.5 ml of supernatant, 2.5 ml of Millipore water, 0.5 ml of 2.5% ammonium molybdate, 0.5 ml of 8-anilino-1-naphthalene sulfonic acid were added and allowed to stand for 10–15 min at 37°C. The released inorganic phosphate was measured at 660 nm using a spectrophotometer [33].

Molecular docking studies

Autodock version 4.2.6 and Autodock Tools version 1.5.6 were used for the docking studies [34–37]. The proteins and ligands used in this study were treated using the united-atom approximation, all water molecules were removed, polar hydrogen atoms were added and Kollman united atom partial charges were allotted. The 3D structure of H^+-K^+-ATPase (protein data bank [PDB] ID: 2XZB) was retrieved from PDB (www.rcsb.org/pdb/) and the ligands (**8a–l**) were docked into the active site of 2XZB.

Membrane protection studies of compounds 8a–l

Inflammation is the first step of ulcer due to the over released gastric substances that are inducing the secretion of lysosomal enzymes by the cellular systems. Cellular activity of these enzymes is known as the acute or chronic inflammation. In fact, the nonsteroidal drugs are acting either by inhibiting the lysosomal enzymes or stabilizing the membrane. Human Red Blood Cells (HRBC) membrane are almost the same as in lysosomal membrane components. Therefore, the inhibition of hypotonicity-induced HRBC membrane lysis was used to measure the membrane protection or anti-inflammatory activity of candidate drugs. In this study, to unveil the potency of **8a–l** on membrane stabilization, HRBC membrane stabilization assay was carried out (n = 4) using Diclofenac as the standard [36,38]. The percentage hemolysis was calculated by using the hemolysis in presence of distilled water at 100% as standard. The percentage of HRBC membrane stabilization was calculated using the following formula:

$$\% \text{ inhibition of hemolysis} = 100 \times [(OD1 - OD2)/OD1]$$

where,

OD2 = optical density of sample

OD1 = optical density of control.

3-(4,5-dimethyl-thiazole-2yl)-2,5-diphenyl tetrazolium bromide assay for antiproliferation/cytotoxicity evaluations

The prolonged and uncontrolled inflammation by action of gastric secretions leads to the development of dysplasia. Having an ulcer increases the risk of developing colorectal (colon) cancer. Initially, we planned to use normal epithelial cell lines to check the cytotoxic effects of **8a–l**. Later, considering the risk factor of ulcer to colon cancer, we also tested the cytotoxicity effect of **8a–l** on colon cancer cell lines. The cytotoxicity assessments were carried out on HCT-116 (colon cancer cell lines) and CCD-18Co (normal colon cells) and determined by the 3-(4,5-dimethyl-thiazole-2yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [34]. Doxorubicin was used as the standard for its wide usage in the treatment of many types of carcinoma and soft tissue sarcomas including blood cancers, like leukemia and lymphoma [39]. Cell viability was calculated using the following formula,

$$\text{Cell viability (\%)} = \text{Mean OD/Control OD} \times 100\%$$

Statistical analysis

All results were expressed as percentage decrease with respect to control values and comparison by one-way analysis of variance (ANOVA) with Dennett's post-test was performed. GraphPad Prism version 7.1 for Windows, GraphPad Software, San Diego, CA, USA (www.graphpad.com) was used for statistical analysis. A difference was considered statistically significant if $p \leq 0.05$. The IC_{50} was calculated from the dose–response curve obtained by plotting percentage inhibition versus concentrations.

Results & discussion

Synthesis of substituted racemic benzimidazoles 8a–l

In this study, compounds **8a–l** (R-enantiomers) were synthesized via asymmetric oxidation. The synthesis of S-enantiomers was not taken into consideration since we were able to obtain R-enantiomers in good yield and with high purity. Impressed from the previous form of **8a–l**, the main purpose of synthesizing the new version of **8a–l** (detaching one carbonyl from –S skeleton [Figure 1]) is only intended to establish them as H^+-K^+-ATPase inhibitors. Since these compounds were predetermined to evaluate as H^+-K^+-ATPase inhibitors, we have designated a synthetic route to achieve the compounds. As described in Figure 1, we have used $(NH_4)_6Mo_7O_{24}.4H_2O/H_2O_2$ as an oxidant for the efficient and selective oxidation of sulfides to sulfoxides [sulfide/50% $H_2O_2/(NH4)_6Mo_7O_{24}.4H_2O$, 1:1.05:0.015 in methanol water]. The efficiency of this method was examined with a range of sulfides as summarized in Table 1.

Table 1. Synthesis and yield percentage details of **8a–l**

Compounds	% of yield	SOR	R1	R2	R3	R4
8a	92	0.001	$-OCH_2CF_3$	-H	$-CH_3$	-H
8b	87	-0.018	$-OCH_2CF_3$	-H	$-CH_3$	$-OCH_3$
8c	85	-0.027	$-OCH_2CF_3$	-H	$-CH_3$	$-OCHF_2$
8d	89	0.007	$-OCH_3$	$-CH_3$	$-CH_3$	-H
8e	79	0.005	$-OCH_3$	$-CH_3$	$-CH_3$	$-OCH_3$
8f	74	0.004	$-OCH_3$	$-CH_3$	$-CH_3$	$-OCHF_2$
8g	45	0.003	$-OCH_3$	-H	$-OCH_3$	-H
8h	72	0.007	$-OCH_3$	-H	$-OCH_3$	$-OCH_3$
8i	47	-0.013	$-OCH_3$	-H	$-OCH_3$	$-OCHF_2$
8j	74	0.008	$-O(CH_{2)3}OCH_3$	-H	$-CH_3$	-H
8k	68	0.007	$-O(CH_{2)3}OCH_3$	-H	$-CH_3$	$-OCH_3$
8l	71	0.015	$-O(CH_{2)3}OCH_3$	-H	$-CH_3$	$-O\ CHF_2$

SOR: Specific optical rotation.

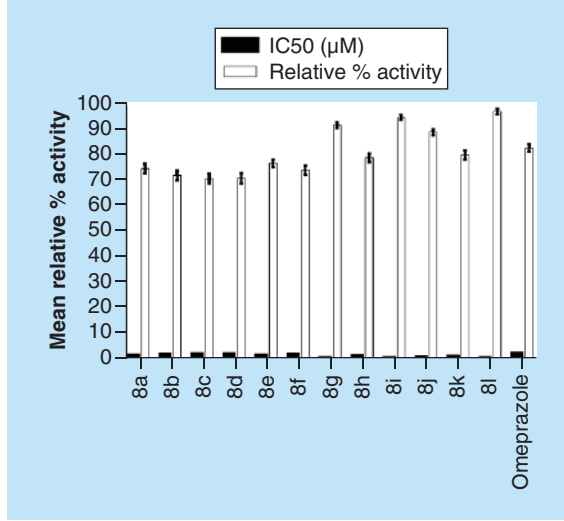

Figure 2. H^+-K^+-ATPase inhibitory potential of benzimidazole derivatives (8a–l). Std* – omeprazole. Note: IC_{50} and H^+-K^+-ATPase inhibitory relative % values are the mean of duplicate/triplicate measurements (n = 4).

Even at increased mole equivalents of H_2O_2 of more than 1.05, there was no impact found on the reaction conversion. On the other hand, the increased mole equivalents of $(NH4)_6Mo_7O_{24}.4H_2O$ up to 0.015 resulted in the overoxidation of **7a–l** that was characterized as the main reason for the formation of anticipated sulfone compounds (**8a–l**). The products were characterized by NMR, FTIR, GC-MS and HRMS. The purity was further analyzed using HPLC. SOR for all these titled sulfoxide compounds was analyzed in 0.5% concentration of methanol. All SOR values are found to be 'zero' that is the expected evidence for the racemic nature of sulfoxides (Table 1; Refer to the Supplementary Information for compound characterization studies and structural confirmation data like NMR, FTIR, SOR, etc.).

H^+-K^+-ATPase inhibition assay results

Figure 2 illustrates the results of gastric H^+-K^+-ATPase inhibition potentials of benzimidazole derivatives (**8a–l**). The released inorganic phosphate was taken as a measurement to estimate the H^+-K^+-ATPase inhibition potentials of the test compounds (**8a–l**). After treating, most reduced orthophosphate released by a test compound was considered as the potent inhibition of H^+-K^+-ATPase. In the results, all compounds were showed significant H^+-K^+-ATPase inhibitory activity in the freshly prepared homogenate from the gastric mucosal of goat ($^*p < 0.05$). Omeprazole, used as the standard drug in this study, is the best example of covalent inhibitors of the pump that is widely used to treat acid-related diseases [40,41]. The obtained inhibitory activity of benzimidazole derivatives (**8a–l**) was dose dependent, and remarkably the results were comparable to omeprazole, the standard drug. Compound **8j** was potentially reduced the hydrolysis of ATP, *in vitro*, by the goat gastric ATPase with IC_{50} of 0.025 μM

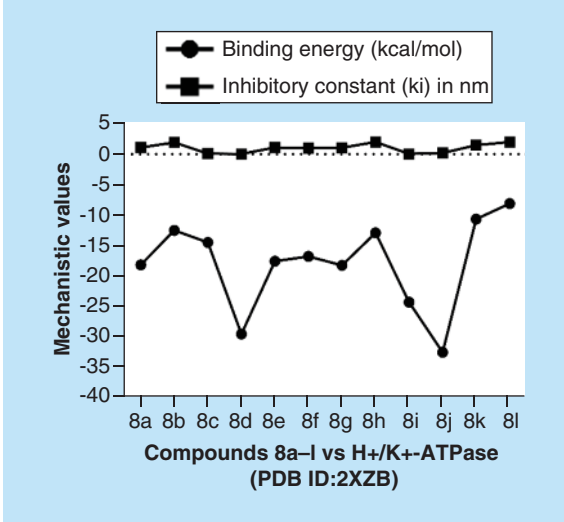

Figure 3. Mechanistic values of molecular docking results. IC_{50} and H^+-K^+-ATPase inhibitory relative % values are the mean of duplicate/triplicate measurements.

(86.12% relative activity). The standard drug, omeprazole (positive control) showed 82.54% relative activity (IC_{50}: 0.95 μM). Overall, the calculated IC_{50} of **8a–l** was in as much as low-molecular volume range of 0.0246 ± 0.08 to 1.95 ± 0.1 μM (the values are averaged from four repeated experimental values and is mentioned as mean ± SD). This signifies the antiulcer therapeutic ability of **8a–l** in low concentrations while comparing to the standard, omeprazole.

Molecular docking studies

To get more insight into the binding mode of **8a–l** into the active sites of H^+-K^+-ATPase (PDB ID: 2XZB), molecular docking was carried out. As depicted in Figure 3, expected molecular docking binding free energy was found between -8.94 and -32.88 kcal/mol and the inhibitory constant (*ki*) range of 0.027–2 μM. The inhibitory constant (*ki*) of a candidate drug (ligands), obtained from *in silico* studies (molecular docking), is the concentration needed to reduce the activity of target enzyme by half by means of inhibition. More precisely, *ki* is the reflective value of binding affinity and the IC_{50} is more reflective of the functional strength of an inhibition potential of a candidate drug. H^+-K^+-ATPase inhibitors (acid-activated thiophilic compounds) substituted with pyridyl-methyl-sulfinyl benzimidazoles are usually constituted with constant disulfide bridges having the key amino acid residues Ala, Lys, Asp and His. These systems are readily accessible from the luminal or acidic surface of the pump.

Subsequently, the interaction between the key amino acid residues (Ala, Lys, Asp and His) was anticipated in the candidate drug. As expected, a significant interaction was found with these amino acid residues. Figure 4 illustrates the fine interaction of compounds **8d** and **8j** (through hydrogen bonds and noncovalent bonds) in the binding pocket of H^+-K^+-ATPase (PDB ID: 2XZB). In general, the presence of hydrogen bond assures the solubility of a chemical entity and the establishment of π–π and cation–π interactions (noncovalent interactions) highly facilitates the positive charge of a cation to bridge to the electrons in a π-system [42,43]. Throughout the molecular docking observations that discussed the molecular interaction of compounds **8a–l** to H^+-K^+-ATPase (PDB ID: 2XZB), a minimum of three hydrogen bond interactions and at least one noncovalent bonding interaction (π–π interaction or π–cation) were found. This indicates the solubility appropriation and the cation bridging to the electrons in the ligand (compounds **8a–l**)-receptor (H^+-K^+-ATPase) interaction.

Results of HRBC membrane stabilization potential of 8a–l

The inhibition potentials of hypotonicity-induced HRBC membrane lysis by **8a–l** were measured by HRBC membrane stabilization assay. This assay results was also taken as a measure to rationalize the nontoxic nature of the compounds against human cellular system. As expected, **8a–l** was showed significant membrane protection activity (Supplementary Table 1). Compounds **8d** (IC_{50}: 0.008 ± 0.02 μM, protection: 92.84 ± 1.02%), **8i** (IC_{50}: 0.045 ± 0.002 μM, protection: 93.22 ± 0.88%), and **8j** (IC_{50}: 0.024 ± 0.002 μM, protection: 98.45 ± 0.84) were significant for the activity in comparison with Diclofenac (the standard [IC_{50}: 0.18 ± 0.012 μM, protection: 88.56 ± 1.04%]).

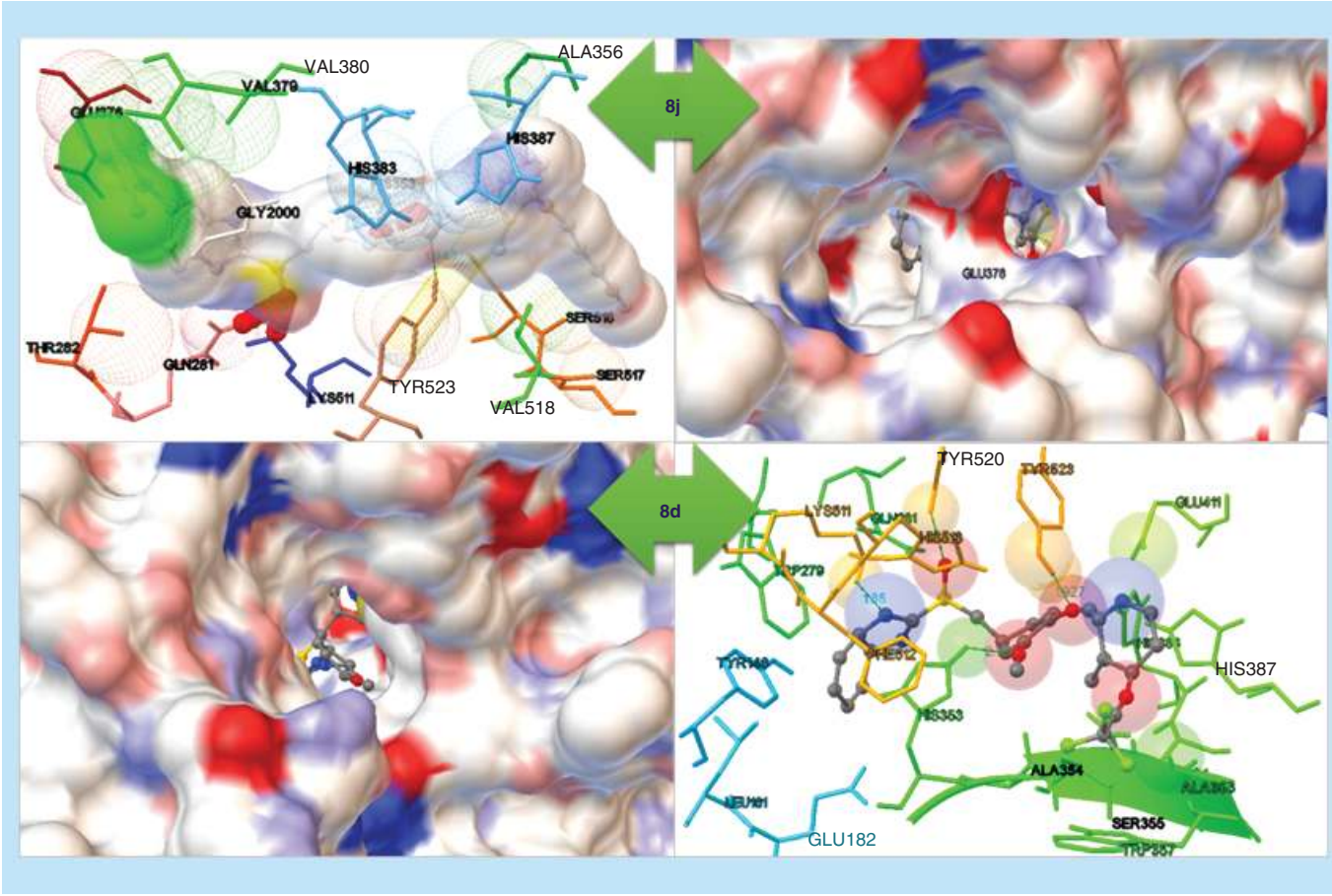

Figure 4. Molecular interaction of compounds 8a–l to H^+-K^+-ATPase (PDB ID: 2XZB). Sticks only: Amino acid residues; Sticks and balls: Compound **8a**; Spherical structures: Hydrogen bond interactions; Solid surface (globular part of the figure): Binding pocket. PDB: Protein data bank.

Cytotoxicity studies: MTT assay

Cytotoxicity study was decisively executed merely to demonstrate the compounds (**8a–l**) safety on the normal epithelial cells. Simultaneously, after recognizing the interrelation between ulcer and colon cancer, the anticancer activity evaluation on normal and colon cancer cells was proposed. Only selected or potentially identified compounds (**8d**, **8i** and **8j**) were analyzed for their cytotoxicity on human colon cancer cell line (HCT-116) and human colon normal cell line (CCD-^{18}Co). Cells were seeded at a density of 1×10^4 cells/well in a 96-well plate and grown for another 24 h. After 24 h of incubation, cell viability was determined by the MTT assay and the inhibitory percentage was calculated (Supplementary Table 2). Compounds **8d** (IC_{50}: 0.12 ± 0.002 μM, % activity 82.96 ± 0.28), **8i** (IC_{50}: 0.12 ± 0.01 μM, % activity 86.12 ± 0.22) and **8j** (IC_{50}: 0.086 ± 0.01 μM, % activity 90.12 ± 0.16) were able to inhibit the proliferation of the cancer cells as active as the standard, doxorubicin (IC_{50}: 0.612 ± 0.012 μM, % activity 80.42 ± 0.20). Figure 5a-d illustrated asthe real-time pictures of human colon cancer cell line (HCT-116) and human colon normal cell line (CCD-18Co) that were treated with the most potent compound **8j**. In the observation up to 8 hours after treating **8j**, there was a remarkable necrosis was observed (Figure 5b) in HCT-116 cell lines due to the drug action (by **8j**) while there was no change in the CCD-18Co cell lines (Figure 5d).

Structure–activity relationships

The anticancer effect of compound **8j** on human colon cancer cell line (HCT-116) and human colon normal cell line (CCD-18Co) can be seen in Figure 5.

In this study, structure–activity relationship (SAR) of racemic-substituted benzimidazoles (**8a–l**) with different electron-withdrawing and electron-releasing groups were analyzed and compared with the H^+-K^+-ATPase inhibition activity and molecular docking interactions. Figure 6 represents the pharmacophores availability of **8a–l**.

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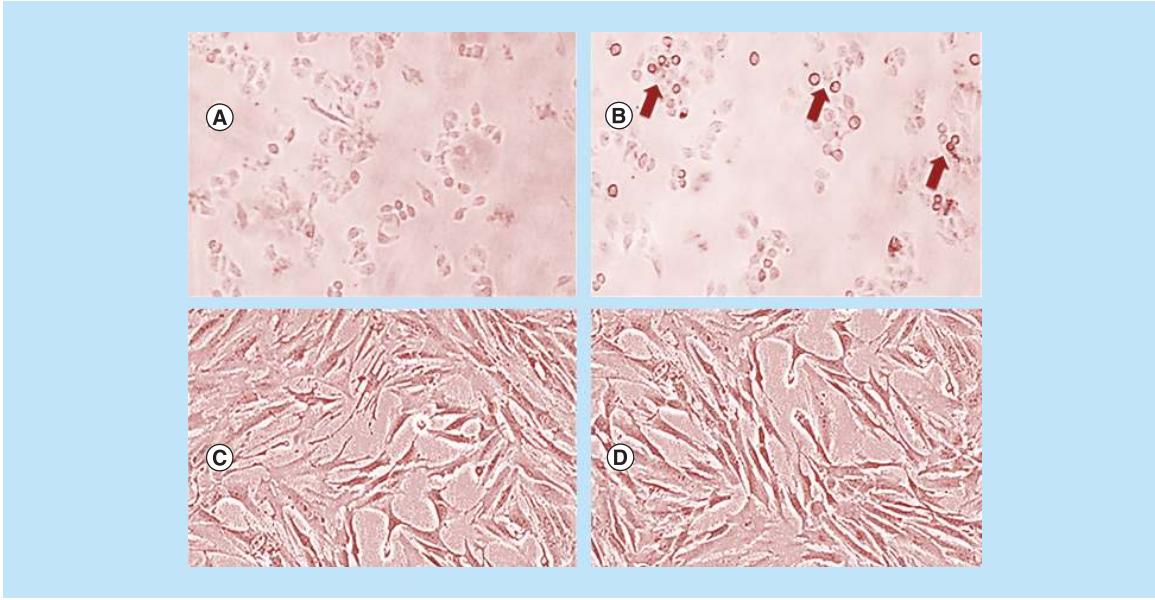

Figure 5. Real-time figures of human colon cancer and human colon normal cell lines before and after (8 h) treating with compound 8j. (A & B) HCT-116; **(C & D)** CCD-^{18}Co.

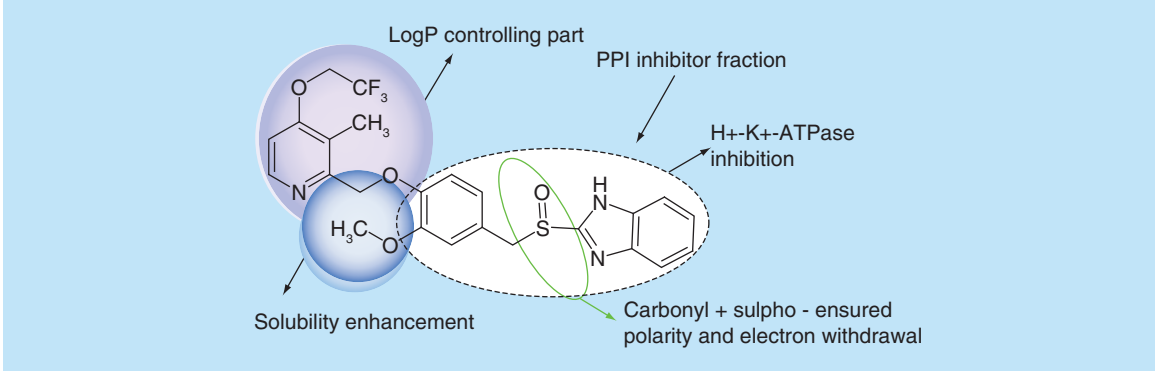

Figure 6. Relative comparison of compounds 8a–l with an established H^+-K^+-ATPase inhibitor.

Omeprazole, one of the most effective H^+-K^+-ATPase inhibitor, is the main axis of **8a–l** that ensures the targeted activity potential. Compounds **8a–h** were substituted with the aromatic arene, heteroarene, azaarene, –CHN containing amines and secondary amines, –CHO containing ether, halogen-containing aryl halides and –S-containing sulfone. All these versatile substituents (of **8a–l**) are facilitated with electron withdrawing and donating groups. These groups are establishing a bridge connection to the receptor and thereby making all the compounds (**8a–l**) potent as the ulcer therapeutic agents. SAR study confirmed a remarkable increase in the potency of **8a–l** that were substituted at the C15–C16 position with the above-mentioned functional groups. Moreover, the antiulcer therapeutic strength of **8a–l** was recognized through the calculated relative % inhibitory activity (range of 60–86%) from the H^+-K^+-ATPase assay. SAR studies based on IC_{50} values of H^+-K^+-ATPase inhibition activity studies also revealed that the compounds **8a–l** are the most suitable candidate drugs to be established as future antiulcer drugs.

Discussion

From its previous form [30], in the present study, compounds **8a–l** were maintained and synthesized with single S-containing sulfone (previously there were two S-containing sulfones were maintained in **8a–l** [Figure 7]) and well established with structural and functional characterizations to establish them as effective ulcer medications. In the present study's chemical reaction, the increased molar equivalents of $(NH4)_6Mo_7O_{24}.4H_2O$ above 0.015,

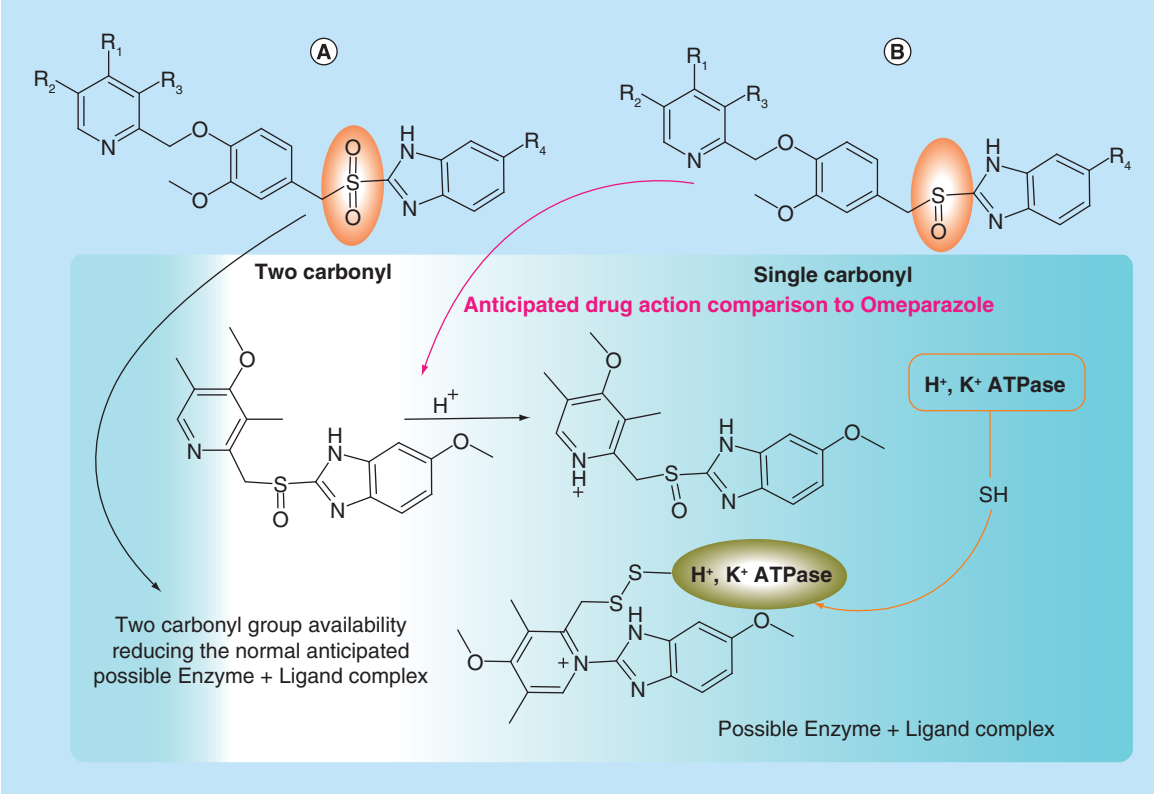

Figure 7. The possible inhibition mechanism of action of 8a-l to H+-K+-ATPase. The illustration of benefit of removal of one carbonyl from structure of earlier reported **(A)** present **(B)** and compounds.

overoxidation lead to the formation of the present study benzimidazole-sulfone compounds (**8a–l**). The oxidation of sulfide to sulfoxide of present study compounds (**8a–l**) was studied to explore the mechanistic details of this highly efficient process, which is one of the industrial-scale catalytic oxidation procedures. Kandasamy *et al.* have reported the use of MoO_2Cl_2/H_2O_2 as an oxidant for the efficient and selective oxidation of sulfides to sulfoxides [sulfide/30% H_2O_2/MoO_2Cl_2, 1:1.05:0.015 in acetone water] [44]. Recently, $(NH4)_6Mo_7O_{24}.4H_2O$ has been used as an efficient catalyst for several organic transformations [45]. This is because the halogen-catalyzed oxidation reaction is sensitive to some functional groups and forms impurities. In an ordinary oxidation reaction, using $VO(acac)_2/H_2O_2$ and $MoO_2(acac)_2/H_2O_2$ more time a mild chilled condition is required for the formation of sulfoxides. When this condition is not controlled, a possible side reaction will take place. Often noticed is that the sulfide oxidation is conveyed by several disadvantages such as long reaction time, problematic reaction conditions and expensive oxidants, undesired side reactions at other functional groups and less yield.

Zirconium chloride and hydrogen peroxides are recently used for the selective oxidation reaction in the conversion of sulfides to sulfoxides. Since the metal halogen catalyzed (H_2O_2/MoO_2Cl_2) oxidation reaction is sensitive to some functional groups and forms impurities, catalytic oxidation using ammonium molybdate tetrahydrate and hydrogen peroxide in aqueous methanol provides controlled oxidation reaction at room temperature yielding substantially pure sulfoxides. At that juncture, racemic-substituted benzimidazoles were designed and synthesized using ammonium molybdate tetrahydrate and hydrogen peroxide in aqueous methanol. This provided controlled oxidation reaction at room temperature yielding substantially pure sulfoxides with the finalized ratio for the reaction as sulfide (50%)$H_2O_2/(NH4)_6Mo_7O_{24}.4H_2O$, 1:1.05:0.015 in methanol water. Furthermore, this reaction does not require any special conditions. Moreover, this catalyst does not oxidize methanol under the designed conditions. In the pharmacological evaluations, significant activity was exhibited by few compounds, but all the compounds were found to inhibit H^+-K^+-ATPase in the least concentrations (IC_{50} range: 0.0246 ± 0.08 to 1.95 ± 0.1 μM). When comparing with the previous version of the compounds (**8a–l**), which are substituted with two carbonyl groups, the present version (with single carbonyl group) was found to have a possible formation of –SH complex (as like the omeprazole mentioned in highlighted part of Figure 7) to the H^+-K^+-ATPase to establish a fortunate

medicinal effect. By the enhanced activity due to the substituted scaffolds on omeprazole axis, the most potent compounds found in this present study could be the alternatives to the available PPIs.

Conclusion

To be concluded, present study aimed to design, synthesis and develop few substituted benzimidazole derivatives as ulcer therapeutics. The proposed synthetic scheme of the present study was found to be an efficient oxidation of sulfides (**7a–l**) to corresponding racemic sulfoxides (**8a–l**) at 20–30°C. This oxidation system is environmentally safe, clean and also it can be carried out with simple unit operations to achieve the compounds in quantitative yields. All these title compounds were significantly showed favorable inhibition activity against H^+-K^+-ATPase. This inhibitory potentials were verified *in silico* by validating the binding affinity and molecular interaction between compounds and target protein. Moreover, these compounds are also able to inhibit the HRBC membrane degradation as well as capable to act as the antiproliferative agents against colon cancer cells. With these assured results, in future, these compounds may be developed as an effective ulcer therapeutics.

Future perspective

In recent era, the search for alternative and novel H^+-K^+-ATPase or PPIs is widely being researched upon. Omeprazole, lansoprazole, pantoprazole and rabeprazole (Figure 1) are the drugs available presently as potent PPIs. Carrying out modifications to these drugs can possibly give us a higher therapeutic value. As observed, addition of methoxy/phenoxy groups to the omeprazole skeleton (**8a–l**) was found to have an improved and significant H^+-K^+-ATPase inhibition making them more effective as medications by means of increased polarity with electron-donating capability. This makes the enhanced bioavailability more conceivably. In such a case, with their biological interactions, benzimidazole-based sulfoxides can be the effective antisecretary agents. Further modifications or substitutions to **8a–l** may correspondingly make these benzimidazole-based sulfoxides as more novel, effective and a resourceful medication. In future, **8a–l** can also be analyzed against other diseases including cancer and inflammation.

Summary points

- Novel, efficient and medicinally important benzimidazoles **8a–l** were discovered as H^+-K^+-ATPase inhibitors.
- *In silico* and *in vitro* evaluations of H^+-K^+-ATPase unveiled to add possible druggability values of these H^+-K^+-ATPase inhibitors.
- The mentioned possible modifications on **8a–l** are the key to execute further chemistry and biological studies.
- Structure–activity relationship studies based on IC_{50} values of H^+-K^+-ATPase inhibition activity studies reveal that the compounds **8a–l** are the most suitable to be established as the antiulcer therapeutics.

Financial and competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of interest

Declared none.

References

Papers of special note have been highlighted as: • of interest

1. Neerja S, Rajni G, Mahendra K, Gupta RR. Synthesis of fluoro phenothiazines via smiles rearrangement and their conversion into sulfones. *J. Fluor. Chem.* 98, 153–157 (1999).

• **Present study targets the synthesis of benzimidazole-based sulfoxides. The basic concept of conversion of target compounds into sulfones in this reference was referred to throughout our work and understood.**

2. Surendra K, Krishnaveni NS, Kumar VP, Sridar R, Rao KR. Regioselective ring opening of oxiranes to halohydrins with tetrabutylammonium halide in water in the presence of β-cyclodextrin. *Lett. Org. Chem.* 2, 652–658 (2005).

- **The authors have worked on the halogen-mediated oxidation to obtain the target compounds. As an example for the usage of halogens to obtain oxidized compounds, this article was cited.**

3. Nenajdenko VG, Gavryushin AE, Baleen ES. Reactions of acceptor thiophene 1,1-dioxides with dienes. Synthesis of bisadducts. *Russ. Chem. Bull.* 54, 2182–2186 (2005).

- **The authors have worked on the halogen-mediated oxidation to obtained dioxide-based compounds.**

4. Velusamy S, Kumar AV, Saini R, Punniyamurthy T. Copper catalyzed the oxidation of sulfides to sulfoxides with aqueous hydrogen peroxide. *Tetrahedron Lett.* 46, 3819–3822 (2005).

- **It is the example for the usage of metal oxides as the oxidation agent. The authors have taken efforts to unveil the synthesis of sulfoxides from sulfides via metal oxide-mediated oxidation processes.**

5. Mckillop A, Tarbin JA. Sodium perborate - a cheap and effective reagent for the oxidation of anilines and sulfides. *Tetrahedron Lett.* 24, 1505–1508 (1983).

- **Reagent-mediated oxidation processes were executed by the authors. The anilines and sulfides are oxidized.**

6. Bahrami K. Selective oxidation of sulfides to sulfoxides and sulfones using hydrogen peroxide (H_2O_2) in the presence of zirconium tetrachloride. *Tetrahedron Lett.* 47, 2009–2012 (2006).

- **The other methodology, using hydrogen peroxide (H_2O_2) in the presence of zirconium tetrachloride as the oxidizing agents, was explained well in this article. This method was found to be one of the effective ways to oxidize sulfides to sulfoxides and sulfones.**

7 . Masayasu K, Yoshio T, Norio I. EP patent: 0302720B1. *Chem. Abstr.* 111, 39369n (1989).

- **References 7–12 provide the extensively cited articles to support the other oxidizing agents available other than the highlighted examples here. $VO(acac)_2/H_2O_2$ [7], $MoO_2(acac)_2/H_2O_2$ [8], NBS/β-cyclodextrin [9], oxone [10] and chromium reagents [11], also for the oxidative cleavage of oximes [12].**

8. Buxade Vinas A. ES patent: 2060541A1; *Chem. Abstr.* 123, 228183f (1995).

9. Surendra K, Krishnaveni NS, Kumar VP, Sridar R, Rao KR. Mild oxidation of alcohols with o-iodoxybenzoic acid (IBX) in water/acetone mixture in the presence of β-cyclodextrin. *J. Org. Chem.*, 68, 2058–2059 (2003).

10. Greenhalgh Richard P. Selective oxidation of phenyl sulfides to sulfoxides or sulphones using Oxone® and wet alumina. *Synlett* **1992, 235–236 (1992).**

24. Patil A, Ganguly S, Surana S. A systematic review of benzimidazole derivatives as an antiulcer agent. *Rasayan. J. Chem.* 1, 447–460 (2008).

- **This comprehensive study summarizes the different derivatives of substituted benzimidazole along with their biological evaluation and factors that make benzimidazole more specific for proton pump inhibitors (PPIs).**

25. Sachs G, Shin JM, Howden CW. Review article: the clinical pharmacology of proton pump inhibitors. *Aliment. Pharmacol. Ther.* 23, 2–8 (2006).

- **It deals with the PPIs that are inhibiting the gastric H^+/K^+-ATPase via covalent binding to cysteine residues of the proton pump. The relative ease with which these steps occur with different PPIs underlie differences in their rates of activation, which in turn influence the location of covalent binding and the stability of inhibition.**

26. Alhazzani W, Alenezi F, Jaeschke RZ, Moayyedi P, Cook DJ. Proton pump inhibitors versus histamine 2 receptor antagonists for stress ulcer prophylaxis in critically ill patients: a systematic review and meta-analysis. *Crit. Care Med.* 41, 693–705 (2013).

- **Critically ill patients may develop bleeding caused by stress ulceration. Acid suppression is commonly prescribed for patients at risk of stress ulcer bleeding. Whether PPIs are more effective than histamine 2 receptor antagonists is unclear. To determine the efficacy and safety of PPIs versus histamine 2 receptor antagonists for the prevention of upper gastrointestinal bleeding in the Intensive Care Unit.**

27. Yuan Y, Ford AC, Khan KJ *et al.* Optimum duration of regimens for *Helicobacter pylori* eradication. *Cochrane Database Syst. Rev.* 12, CD008337 (2013).

- **The optimal duration for *Helicobacter pylori* eradication therapy is controversial, with recommendations ranging from 7 to 14 days. Several systematic reviews have attempted to address this issue but have given conflicting results and limited their analysis to PPI, two antibiotics (PPI triple) therapy. We performed a systematic review and meta-analysis to investigate the optimal duration of multiple *H. pylori* eradication regimens. The primary objective was to assess the relative effectiveness of different durations (7, 10 or 14 days) of a variety of regimens for eradicating *H. pylori*. The primary outcome was *H. pylori* persistence. The secondary outcome was adverse events.**

28 . van Pinxteren B, Numans ME, Bonis PA, Lau J. Short-term treatment with proton pump inhibitors, H2-receptor antagonists and prokinetics for gastro-oesophageal reflux disease-like symptoms and endoscopy-negative reflux disease. *Cochrane Database Syst. Rev.* 19, CD002095 (2006).

- **It summarizes, quantifies and compares the efficacy of short-term use of PPIs, H2-receptor antagonists (H2RA) and prokinetics in adults with gastro-oesophageal reflux disease, treated empirically, and in those with endoscopy negative reflux disease.**

29. Scarpignato C, Gatta L, Zullo A, Blandizzi C. Effective and safe proton pump inhibitor therapy in acid-related diseases – a position paper addressing benefits and potential harms of acid suppression. *BMC Med.* 14, 179 (2016).

- **It concludes that PPIs are irreplaceable drugs in the management of acid-related diseases. However, PPI treatment, as with any kind of drug therapy, is not without risk of adverse effects. The overall benefits of therapy and improvement in quality of life significantly outweigh potential harms in most patients, but those without clear clinical indication are only exposed to the risks of PPI prescription. Adhering with evidence-based guidelines represents the only rational approach to effective and safe PPI therapy. Please see related commentary: doi: 10.1186/s12916-016-0724-1**

30. Rajesh R, Manikandan A, Sivakumar A, Ramasubbu C, Nagaraju N. Substituted methoxybenzyl-sulfonyl-1H-benzo[d]imidazoles evaluated as effective H^+-K^+-ATPase inhibitors and anti-ulcer therapeutics. *Eur. J. Med. Chem.* 139, 454–460 (2017).

- **Efforts were made by the authors to synthesize a series of substituted methoxybenzyl-sulfonyl-1H-benzo[d]imidazole derivatives (8a–l) and investigate their antiulcer therapeutics. Prior to evaluating antiulcer potentials of 8a–l, a preliminary binding assay against H^+/K^+-ATPase from goat gastric mucosa was carried out, since it plays an important role in the ulcer development.**

31. Sachs G, Spenney JG, Lewin M. H+ transport: regulation and mechanism in gastric mucosa and membrane vesicles. *Physiol. Rev.* 58, 106–173 (1978).

- **A review article discussing about H^+ transport: regulation and mechanism in gastric mucosa and membrane vesicles.**

32. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254 (1976).

- **A protein determination method which involves the binding of Coomassie Brilliant Blue G-250 to protein is described. The binding of the dye to protein causes a shift in the absorption maximum of the dye from 465 to 595 nm, and it is the increase in absorption at 595 nm, which is monitored. This assay is very reproducible and rapid with the dye-binding process virtually complete in approximately 2 min with good color stability for 1 h.**

33. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. *J. Biol. Chem.* 66, 375–400 (1925).

- **H^+, K^+ ATPase inhibition assay will be facilitated by measuring the released inorganic phosphate at 660 nm using a spectrophotometer. It was useful in this article.**

34. Alagumuthu M, Arumugam S. Molecular explorations of substituted 2-(4-phenyl quinoline-2-yl) phenols as phosphoinositide 3-kinase inhibitors and anticancer agents. *Cancer Chemother. Pharmacol.* 79, 389–397 (2017).

35. Sridhar P, Manikandan A, Sivakumar A, Reddy SR. Synthesis of quinoline acetohydrazide-hydrazone derivatives evaluated as DNA gyrase inhibitors and potent antimicrobial agents. *RSC Adv.* 6, 64460–64468 (2016).

- **References [34] and [35] were cited by referring molecular docking study.**

36. Manikandan A, Sivakumar A. Molecular docking, discovery, synthesis, and pharmacological properties of new 6-substituted-2-(3-phenoxyphenyl)-4-phenyl quinoline derivatives; an approach to developing potent DNA gyrase inhibitors/antibacterial agents. *Bioorg. Med. Chem.* 25, 1448–1455 (2017).

- **It was referred to molecular docking study, especially to unveil the structure–activity relationship studies using the *in silico* predictions.**

37. Manikandan A, Ravichandran S, Kumaravel K, Sivakumar A, Rethna P. Molecular docking and *in vitro* evaluations of *Hippocampus trimaculatus* (seahorse) extracts as the anti-inflammatory compounds. *Int. J. Bioinform. Res. Appl.* 12, 355–371 (2016).

- **References [37] and [38] were referred to molecular docking study and HRBC membrane stabilization assay.**

38. Alagumuthu M, Ravichandran S, Sathiyanarayanan KI, Sivakumar A. Efficacy of phenyl quinoline phenol derivatives as COX-2 inhibitors; an approach to emergent the small molecules as the anti-inflammatory and analgesic therapeutics. *Inflammopharmacology* 25(6), 621–631 (2017).

39 . Shrivastava N, Naim MJ, Alam MJ, Nawaz F, Ahmed S, Alam O. Benzimidazole scaffold as anticancer agent: synthetic approaches and structure–activity relationship. *Arch. Pharm. (Weinheim)* 350, doi:10.1002/ardp.201700040 (2017).

- **It covers the current development of benzimidazole-based anticancer agents along with the synthetic approaches and structure–activity relationships. Benzimidazole is an organic compound that is heterocyclic and aromatic in nature. It is a bicyclic compound formed by the fusion of the benzene and imidazole ring systems. It is an important pharmacophore and a privileged structure in medicinal chemistry. In the efforts to develop suitable anticancer drugs, medicinal chemists have focused on benzimidazole derivatives.**

40. Sachs G, Chang HH, Rabon E, Schackman R, Lewin M, Saccomani G. A non-electrogenic H+ pump in plasma membranes of hog stomach. *J. Biol. Chem.* 251, 7690–7698 (1976).

    It discusses about the differential and density gradient centrifugation that were used to prepare a vesicular membrane fraction from hog gastric mucosa enriched 17-fold with respect to cation-activated ATPase and 5′-AMPase. The ionophoric and osmotic sensitivity showed that these ion movements were due to transport rather than binding. The cation selectivity sequences, substrate specificities and action of inhibitors indicated that the transport was a function of K^+ ATPase activity.

41. Sachs G, Shin JM, Besancon M, Prinz C. The continuing development of gastric acid pump inhibitors. *Aliment. Pharmac. Ther.* 7, 4–12 (1993).

- **The synthesis and action of H2RA changed the understanding of gastric acid secretion as well as changing medical therapy for peptic ulcer disease. There is no final common pathway for stimulation of the parietal cell. In contrast, all stimuli converge to activate the acid pump, the H^+, $K^{(+)}$-ATPase. Inhibition of this pump by clinically useful drugs was achieved by developing**

derivatives of timoprazole, pyridyl-2-methylsulfinyl benzimidazole. Two of these derivatives, omeprazole and lansoprazole, have shown superiority in acid control and therefore in therapy for peptic ulcer disease compared with the available H2RA.

42. Anslyn E, Sausalito CA. *Modern Physical Organic Chemistry*. University Science Books, VA, USA, 1104 (2004).

- **References 42 and 43 deal with noncovalent interactions in biochemical intermediates and their uses in drug interactions to host system. The presence of hydrogen bond guaranteeing the solubility of a chemical entity and the establishment of π–π and cation–π interactions (noncovalent interactions) are highly facilitating the positive charge of a cation bridging to the electrons in a π-system**

43. Scott LC, Christopher AH. Chemical double-mutant cycles: dissecting noncovalent interactions. *Chem. Soc. Rev.* 36, 172–188 (2007).

44. Kandasamy J, Rajan DC, Dillip KC. A simple and efficient method for the oxidation of sulfides to sulfones using hydrogen peroxide and a Mo(VI) based catalyst. *Catal. Commun.* 10, 1948–1951 (2009).

- **A simple and efficient method for the oxidation of sulfides to sulfones at room temperature using ammonium heptamolybdate and 30% H_2O_2 is developed. The reactions provide excellent yields within a short time, and also sensitive functional groups such as allyl, vinyl, propargyl, alcohol, ketone, ester, pyridine and nitrile are found to be tolerated.**

45 . Javad S, Shiva DK, Sayed HB. A novel and an efficient catalyst for one-pot synthesis of 2,4,5-trisubstituted imidazoles by using microwave irradiation under solvent-free conditions. *J. Chem. Sci.*122, 437–441 (2010).

- **$(NH_4)_6Mo_7O_{24}·4H_2O$ has been used as an efficient catalyst for an improved and rapid synthesis of 2,4,5-trisubstituted imidazoles by a three-component, one-pot condensation of benzil, aryl aldehydes and ammonium acetate in good yields under solvent-free conditions using microwave irradiation. The reactions in conventional heating conditions were compared with the microwave-assisted reactions.**