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Review article

Therapeutic significance of β-glucuronidase activity and its inhibitors: A review

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ABSTRACT

The emergence of disease and dearth of effective pharmacological agents on most therapeutic fronts, constitutes a major threat to global public health and man's existence. Consequently, this has created an exigency in the search for new drugs with improved clinical utility or means of potentiating available ones. To this end, accumulating empirical evidence supports molecular target therapy as a plausible egress and, β-glucuronidase (βGLU) – a lysosomal acid hydrolase responsible for the catalytic deconjugation of β-D-glucuronides has emerged as a viable molecular target for several therapeutic applications. The enzyme's activity level in body fluids is also deemed a potential biomarker for the diagnosis of some pathological conditions. Moreover, due to its role in colon carcinogenesis and certain drug-induced dose-limiting toxicities, the development of potent inhibitors of βGLU in human intestinal microbiota has aroused increased attention over the years. Nevertheless, although our literature survey revealed both natural products and synthetic scaffolds as potential inhibitors of the enzyme, only few of these have found clinical utility, albeit with moderate to poor pharmacokinetic profile. Hence, in this review we present a compendium of exploits in the present millennium directed towards the inhibition of βGLU. The aim is to proffer a platform on which new scaffolds can be modelled for improved βGLU inhibitory potency and the development of new therapeutic agents in consequential.

Contents

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1. Introduction

The world today is embattled with an increasing paucity of effective therapeutic agents or regimen for many pathological conditions, as well as the menace of drug resistance and adverse effects of available drugs [1]. As a result, smooth and efficient clinical practice is rigidly stymied, while global public health, social security and man's life expectancy are seriously threatened and trickles to a disquieting edge [2]. Likewise, the burdens of developing new therapeutic agents to ameliorate the status quo has become heavier on all stakeholders in drug research.

In this regard, molecular target therapy is fast becoming a spearhead in the search for new drugs with improved therapeutic effects. Amongst many targets explored, glycosyl hydrolases (GHs) are notable due to their role in many important biological processes. Their principal function is to catalytically cleave the glycosidic bond of glycans thereby eliciting different physiological responses. Therefore, inhibitors of this class of enzymes have enjoyed intense research and development owing to their potentials as antiviral, anticancer and antidiabetic agents as well as therapeutic agents for some genetic disorders [3–5].

GHs have been classified using different indices [6]. For example, based on substrate specificity, those cleaving *O*- or *S*-glycosides are grouped into EC 3.2.1 class, while hydrolases of *N*-glycosides belong to EC 3.2.2 class. Advancements in genomic science have also enabled classification into GH families based on their amino acid sequence similarities [7]. This system further groups GH families into clans, given the improved conservation of protein fold than the sequence [8]. Accordingly, the reviewed enzyme, β-glucuronidase (EC 3.2.1.31) is classified into GH family 1, 2, 30, 79, 154 and GH-A clan. β-glucuronidase (βGLU) is mainly a lysosomal hydrolase widely distributed in mammalian tissues, body fluids and microbiota; but significantly retained in the endoplasmic reticulum [9]. The enzyme is also found in plants, fishes, insects and molluscs.

Specifically, human βGLU belongs to GH family 2. It is a 332 kDa ellipsoidal and homotetrameric glycoprotein with each 75–78 kDa monomer containing 651 amino acid residues (Fig. 1a). The monomer precursor is synthesized initially on membrane-bound ribosomes and suffers *C*-terminal proteolytic processing of 18 amino acid propeptide *en route* or after their transport to the lysosomes [10–13]. X-ray crystallography of the protein structure reveals a dihedral symmetry for the tetramer with two identical monomers in the asymmetric unit arising from disulphide-linked dimers. Each monomer contains three structural domains (Fig. 1b). The first domain has a barrel-like structure with a jelly roll motif; the second domain exhibits a geometry identical to immunoglobulin constant domains; while the third *C*-terminal domain forms a TIM barrel motif $(\beta/\alpha)_8$ [14]. The active sites of human βGLU (Fig. 1c) *viz*. catalytic acid Glu451 (proton donor), catalytic nucleophile Glu540 (carbonium ion stabilizer), Asp207 (plausible role as Glu540) and Tyr504 (unclear catalytic role), are all housed in the third domain and in each of the four catalytic centres of the tetramer [14,15]. Moreover, the enzyme has an optimal activity at acidic pH ~4.5, corresponding to its lysosomal environment and thermally stable up to 70 °C [10]; although hyperthermophilic variants exists in other media [16]. βGLU is encoded by the GUS gene. A deficiency arising from mutations in this encoding gene is associated with atherosclerosis [17] and lysosomal storage disease – Sly syndrome or mucopolysaccharidosis type VII [18].

On the other hand, bacterial βGLU, which is expressed in human gut microbiota and most strains of *Escherichia coli* shows 45% sequence similarity with human βGLU. Also, it has a bacterial loop containing 17-amino acid residues not found in human βGLU, an optimal activity at neutral pH and active site catalytic residues as Glu413 (catalytic acid) and Glu504 (catalytic nucleophile) [19].

Consistent with the activities of lysosomal GHs, βGLU deconjugates β-D-glucuronides to their corresponding aglycone and β-D-glucuronic acid *via* an S_N2 reaction and "configuration retaining" mechanism (Fig. 2). The catalytic mechanism is conceived to proceed as follows; catalytic glutamic acid residue Glu451 (or Glu413 in bacterial ortholog) protonates exocyclic glycosidic oxygen of glucuronide (**1**) hence releasing the aglycone *via* a putative oxocarbenium ion-like transition state (**2**). 'Back-side' nucleophilic attack by glutamate ion Glu540 (or Glu504 in bacterial ortholog) – the catalytic nucleophile, stabilizes the transition state and results in glucuronyl ester intermediate (**3**) with an inverted configuration. Finally, hydrolysis through an inverting attack of water molecule on the anomeric centre releases Glu540 to form β-D-glucuronic acid (**4**) and a concurrent overall retention of substrate configuration [14,15,19–21].

Due to the increased expression of βGLU in necrotic areas and other body fluids of patients with different forms of cancer such as breast [22], cervical [23], colon [24], lung [25], renal carcinoma and leukaemia [26], compared to healthy controls, the enzyme is proffered as a reliable biomarker for tumour diagnosis and clinical therapy assessment [27]. This overexpression is also a potential diagnostic tool for other disease states such as urinary tract infection [28], HIV [29], diabetes [30], neuropathy [31] and rheumatoid arthritis [32]. In this vein, empirical data update on clinical applications of βGLU for these and other disorders is provided on BRENDA database [33].

βGLU activity is also harnessed in prodrug monotherapy. In normal body systems, drugs and other xenobiotics are detoxified *via* glucuronidation, an S_N2 conjugation reaction and important pathway in phase II metabolism, catalysed by UDP-

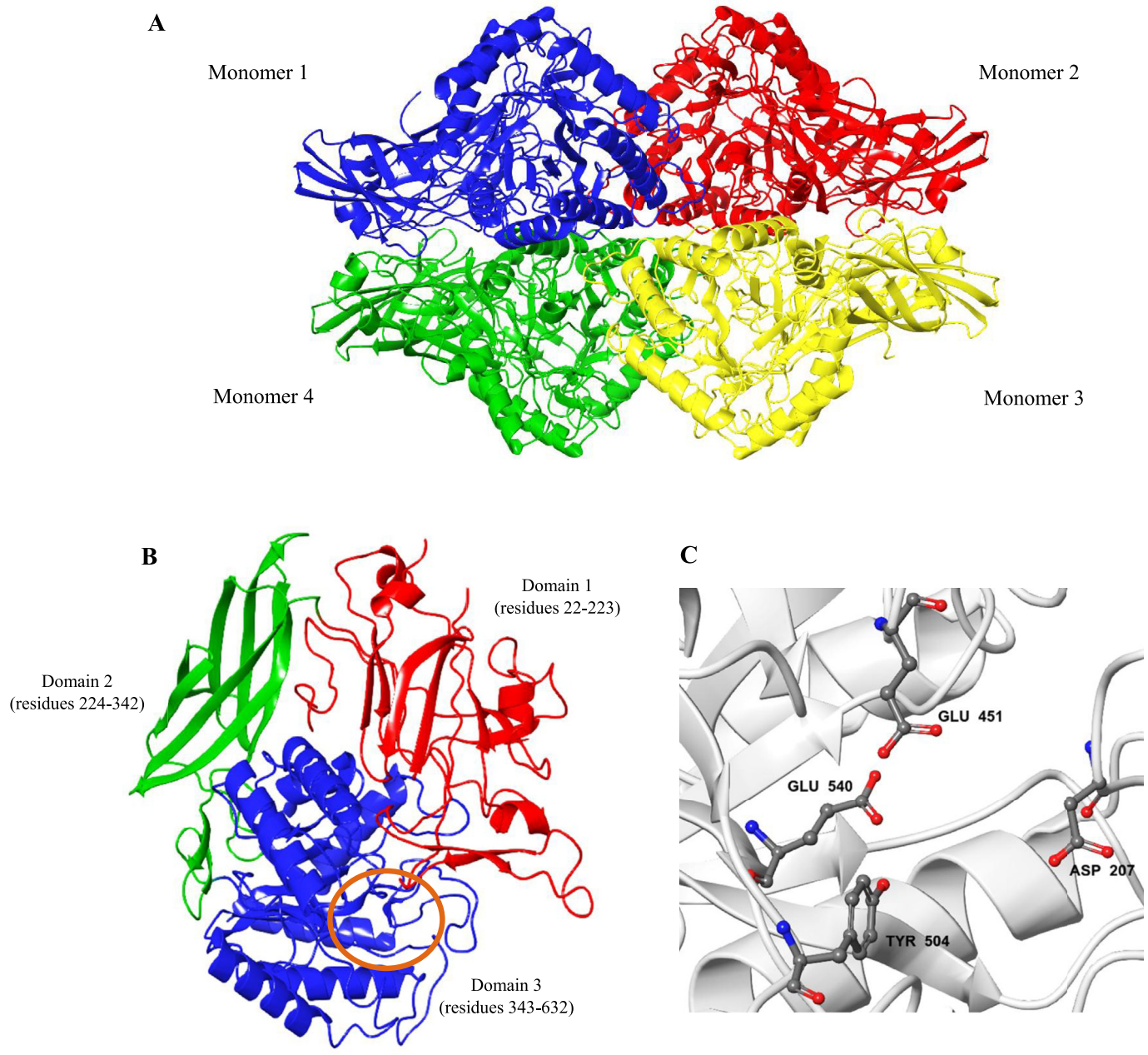


Fig. 1. 3D ribbon diagram of human βGLU (**A**) Homotetramer (PDB ID: 3HN3) (**B**) Monomer structure showing the three structural domains and active site cavity (brown ring). (**B**) Expanded view of active site cavity. Protein structure was processed using Maestro 12.0. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

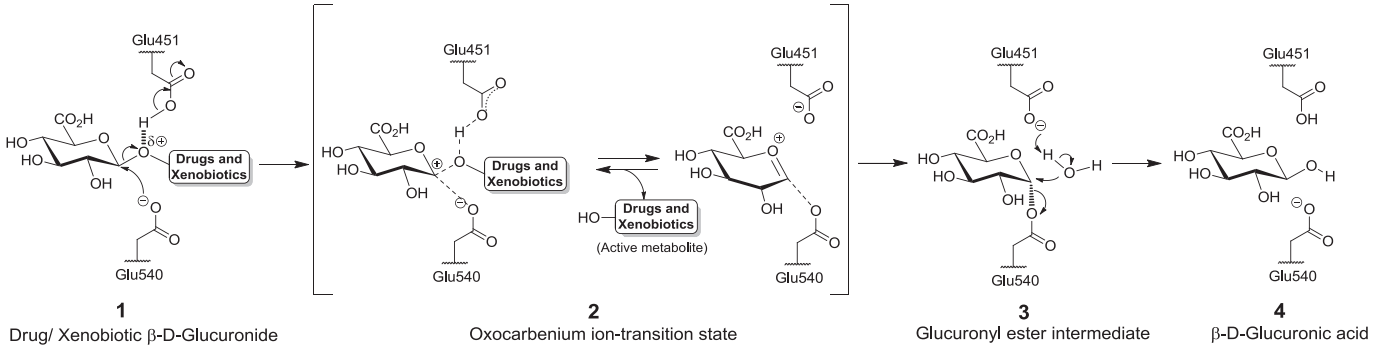


Fig. 2. Configuration retaining mechanism of βGLU catalysed hydrolysis.

glucuronosyltransferases (UGTs). The resulting usually less active glucuronide metabolite is readily excreted by renal clearance due to increased polarity or sometimes *via* biliary clearance [34]. However, elevated levels of βGLU activity reverts this process through deglucuronidation, which hydrolyses the phase II metabolites to their active forms (Fig. 2). Hence, glycosidation of a drug to give its glucuronide enhances selective release of the active form at necrotic sites *via* βGLU-mediated deglucuronidation thus improving the drug's therapeutic potential [35].

βGLU's postulated ability to increase T Regulator cells (TReg) is also applied in low-dose immunotherapy (LDI) for managing allergic diseases [36,37], Lyme disease [38] and other chronic conditions. While it's hydrolytic activity on glucuronide conjugates is harnessed in forensic analysis [39] and assessment of microbial water quality [40].

Nonetheless, enterobacterial βGLU deconjugation of drug and xenobiotic glucuronides in the gastrointestinal (GI) tract has been implicated in colonic genotoxicity [41] and certain drug-induced-dose-limiting toxicities. For example, the GI toxicity of anticancer drug Irinotecan (CPT-11) [42], enteropathy of non-steroidal anti-inflammatory drug (NSAID) Diclofenac [43], tissue inflammation and hepatoxicity.

Furthermore, βGLU is deemed a potential molecular target for; (1) anticancer chemotherapy considering its role in tumour growth and metastasis [44,45]. (2) Neonatal jaundice treatment due to its high expression in breast milk and role in enterohepatic bilirubin circulation (hyperbilirubinemia) [46,47]. (3) Diabetes mellitus management consequent to the positive correlations between the disease state and enzyme activity level as well as associated periodontitis [48,49]. (4) Anti-inflammatory agents development owing to its pro-inflammatory role following significant release from degranulated mast cells and neutrophils [50,51]. Expectedly, inhibition of βGLU markedly alleviated these pathological conditions and their adverse effects hence improving regimens' efficacy.

Based on the foregoing, we extrapolate that the development of potent, specific and non-cytotoxic inhibitors of βGLU is imperative to improving the clinical efficacy of therapeutic agents and effective disease management while bearing in mind the physiological significance of both human and bacterial orthologs of the glycosyl hydrolase. However, the fate of these inhibitors rests on their inhibition constants (K_i), since GHs are generally characterized by high rate enhancement ($k_{cat}/k_{uncat} > 10^{17}$-fold). Also, accumulating evidence suggests the dependence of inhibitory potency on the ability to mimic the highly enzyme-stabilized transition state of an enzymatic reaction ($K_i \approx 10^{-20}$ M) *en route* to catalytic product [21,52,53].

Considering the proven and encouraging potentials of enzyme inhibition and molecular target therapy in drug development, and in continuation of our exploits and expositions thereon [54–58], herein we present a comprehensive review of research undertakings in the present millennium (2000–2019) directed towards the development of potent inhibitors of βGLU that are either natural products or synthetic scaffolds. Apropos, before discussing the different inhibitors, this article will first highlight the potentials of βGLU activity as a diagnostic tool within the defined period. However, therapeutic application in prodrug monotherapy and enzyme replacement therapy (ERT) will not be covered as these have been excellently treated in other reviews [59–61].

Hitherto, our search of extant literature revealed that, although there exists a plethora of scholarly research on potential inhibitors of βGLU activity, no review article is exclusively devoted to the subject matter. The aim of this review is therefore to bring to light those bioactive frameworks bestowed with promising βGLU inhibitory potency. Our principal goal is to intimate the reader on key structural features of reviewed molecules crucial to their inhibitory activities and toxicity profiles, while establishing the comprehensive relationships existing between reported molecules.

2. β-Glucuronidase activity as a reliable biomarker in diagnostic science

The availability of safe, easy to use, consistent, less-invasive and cost-effective tool for early diagnosis of diseases or appraisal of therapeutic interventions is of uttermost importance in clinical medicine. Since most disease states are accompanied by elevated levels of specific enzymes in the diseased milieu (tissues, plasma and other body fluids), quantification of enzymes' activity levels is seen as a reliable biomarker of either disease status, severity, effects, susceptibility or exposure [62–64]. Moreover, the substrate specificity and selective quantification of enzymes in the presence of other biomolecules makes them a tool of choice thereto [65]. A review of βGLU activity as a biomarker of some physiologically important conditions is hereby presented together with a concise summary in Table 1.

2.1. Periodontal disease

Periodontal disease is a group of inflammatory disorders triggered by host's immune response to the actions of virulent subgingival plaque bacteria biofilms which activates the release of polymorphonuclear leukocytes and macrophages into the gingival crevice. This leads to gingivitis – an inflammation of periodontal tissues and distortion of periodontal histology that is reversible with improved oral hygiene; or, subsequent tissue destruction, alveolar bone resorption and tooth loss if left unattended *i.e.* periodontitis [66,67]. Therefore, a reliable tool to ascertain disease status, severity, risk or efficacy of administered therapy is highly desirous to clinicians.

However, conventional diagnosis involving the measurement of periodontal clinical parameters such as probing depth (PD), clinical attachment level (CAL), gingival index (Gg-I), bleeding on probing (BOP) and alveolar bone loss (ABL), suffers from intrinsic limitations. They only define the status of patient's periodontium at the time of examination and not periodontal disease susceptibility or risk [68]. Thus, since periodontitis is characterized by an influx of inflammatory mediators and corresponding enzymes into the gingival sulcus, the quantification of neutrophil-derived βGLU activity in gingival crevicular fluid (GCF) or GCF's outflow into the oral cavity and subsequent less invasive estimation of βGLU activity in saliva is considered a reliable biomarker for periodontal disease diagnosis.

To this effect, the relationship between salivary βGLU activity and periodontal clinical parameters (PD, CAL and Gg-I) was investigated in subjects with different stages of periodontal disease [69]. The mean PD and Gg-I, number of sites with PD $\geq$ 5 mm and total number of white blood cells, blood neutrophils and monocytes all showed highly significant correlations with enzyme's activity, while CAL had a weaker correlation. Using logistic regression modelling and the presence of at least 1 or 4 sites with PD $\geq$ 5 mm as disease criterion, βGLU activity showed promising potentials as a tool for periodontal disease screening or assessment of therapeutic intervention. The study also observed smoking status to be insignificant on enzyme activity. However, in a similar study, only PD, CAL and lymphocyte count exhibited positive correlation with salivary enzyme activity while no significant relationship was observed for Gg-I [70]. Recently, subjects with chronic generalized periodontitis were also found to have significant increase in βGLU activity (8-fold) compared to normal ones. Although, a reduction in enzyme activity persisted in smokers regardless of periodontal status [71].

Table 1
Reported potential applications of βGLU activity as a biomarker.

Pathological Condition	Significance of βGLU activity	Media	Study size	Parameter with significant correlation to βGLU activity	βGLU activity in diseased subjects compared to controls	Required βGLU activity	Ref
Periodontal Disease	Biomarker of • oral inflammation • disease risk and • disease severity	Saliva	380	• Mean PD • Mean Gg-I • Number of sites with PD ≥ 5 mm • CAL (W) • Blood's total number of - white blood cells - neutrophils and - monocytes	• Elevated	≥100 units	[69]
	Biomarker of disease susceptibility		70	• PD • CAL • Lymphocyte count		˃ 100 units	[70]
			200	NS		NS	[71]
	Biomarker of disease status	GCF	14	• GCF volume • PD • CAL • Gg-I			[72]
	Biomarker of disease severity and assessment of non-surgical therapy	Saliva	31	NS			[73]
Diabetes and Periodontitis	Biomarker of disease risk		80	• PD • CAL	• Elevated in all • Highest in diabetic subjects		[86]
	Biomarker of disease status and severity	Serum	350		• Elevated in all • Highest in periodontitis subjects		[87]
		Peripheral venous blood neutrophilic leukocytes	165		• Elevated in all • Highest in diabetic subjects		[88]
		Saliva	192	NS	• Elevated in all		[89]
		GCF	45	• PD • CAL • BOP	• Highest in periodontitis subjects		[90]
Colon Cancer	Tumour biomarker	Serum	38	• Clinical grading • Cell maturity	• Elevated	>208.10 pKat/mL	[96]
Ovarian and Endometrial Cancer	Biomarker of tumour status and severity	Peritoneal fluid	35	• Cancer Stage	• Elevated in all • Highest in patient with stage IV cancer	NS	[97]
Pelvic inflammatory disease	Biomarker of disease status			NS	• Higher in patients with ruptured tubo-ovarian abscess compared to those with acute salpingitis		
Bacterial peritonitis	Biomarker for early disease diagnosis and assessment of therapeutic intervention		71		• Elevated in all regardless of the origin of peritoneal inflammation • Higher in patients with culture positive bacterial peritonitis		[98]
Bacterial meningitis		CSF	140		• Elevated		[99]
Sterile CSF pleocytosis due to UTI or meningitis	Biomarker for differential diagnosis		92		• Elevated in all • Highest in neonates (≤3 months old) with bacterial meningitis		[100]
Bacterial lung infection	Biomarker for disease diagnosis, prognosis and differential BALF bacterial culture screening	BALF	92	BALF levels of • TNF-α • IL-8	• Elevated in all • Higher in subjects with positive BALF bacterial culture • Highest in subjects with recurrent pneumonia	*43 nmol 4MU/ml/h	[101]
Organophosphorus pesticide poisoning	Biomarker of poisoning severity	Plasma	74	NS	• Elevated in all	NS	[108]
			108	• BuChE activity • AcP activity	• Higher in severely poisoned subjects		[109]
		Serum	21	NS			[110]
			40	• BuChE activity	• Elevated in all		[111]
		Plasma	220	• BuChE activity • AChE activity (N) • Blood glucose • Total lipids • Triglyceride • Cholesterol • Lipoproteins • Liver function parameters	• Highest in mildly poisoned subjects		[112]
			284**	AChE activity • Chronic exposure (W) • Acute exposure (N)	• Elevated in 16.5% and 60% of subjects with chronic and acute exposure respectively		[113]

NS: Not specified; W: weak correlation; N: No correlation; PI: Russell periodontal index; *threshold to distinguish culture positive from culture negative BALF; ** acute exposure (5), chronic exposure (230).

The efficacy of therapeutic intervention using amoxicillin and metronidazole to downregulate amplified neutrophil activity was studied in 14 patients with aggressive periodontitis [72]. Treatment involved seven consecutive days of antibiotic administration with concurrent scaling, root planning and surgical therapy and a total of 36 months posttreatment evaluation period. Subsequently, a markedly downregulated neutrophil activity with approximately 50% inhibition of βGLU activity in GCF was observed. Periodontal health was also restored and maintained during posttreatment evaluations. In another study, βGLU activity was posited as a better biomarker compared to alkaline phosphatase for evaluating the response to non-surgical periodontal therapy in patients with different stages of periodontal disease [73]. Taken together, these results articulate the potentials of βGLU activity as an indication of PD, tissue inflammation or destruction as well as a biomarker of neutrophil influx, disease risk, susceptibility, status, or severity for timely diagnosis of the inflammatory disorder. However, administration of doxycycline hyclate in 16 subjects with aggressive periodontitis was inefficient on salivary βGLU activity [74]. Surprisingly, an increase in enzyme activity was found even after 2 months of treatment in 12 patients and a decrease in 4. Although, the authors concluded βGLU concentration only facilitated the detection of periodontal inflammation and not worthy as biomarker of susceptibility, their contrasting result is linkable to periodontal pretreatment of subjects prior to examination and short treatment time using doxycycline.

Empirical evidence affirms the role of inflammatory mediators and signalling pathways in the pathogenesis of insulin resistance and β-cell dysfunction in diabetes mellitus [75–77]. In parallel, these inflammatory mediators e.g. cytokines and MMPs are also produced in periodontal tissues [78–82]; hence, leading to compromised glycaemic control after accessing systemic circulation. The susceptibility to periodontitis is therefore heightened in persons with diabetes or a history of the hormonal imbalance and *vice versa*. Putatively, an effective therapy for one affords an improved management of the other [83–85].

Accordingly, βGLU activity was found to be significantly elevated in the saliva of patients with chronic periodontitis and diabetes compared to nondiabetic ones [86]. A significant correlation to βGLU activity was observed for PD and CAL and not Gg-I in nondiabetic subjects with periodontitis; whereas, these periodontal parameters were similar in diabetics. The increased disease burden was also established when βGLU activity was measured in the sera of patients with both diabetes and periodontitis [87]. Compared to controls, enzyme activity was 9-fold higher in diabetic subjects with periodontitis and only 2-fold higher in diabetic subjects without periodontitis. This difference was attributed to damaged lysosomal membrane and consequent enzyme leakage into the cytosol.

The quantification of βGLU activity in neutrophil leukocytes exposed to bacteria stimuli has shown that diabetic patients with chronic periodontitis have strikingly higher enzyme activity compared to nondiabetics burdened with periodontitis and healthy subjects [88]. Using discriminant analysis, the study established that βGLU activity has a diagnostic potential with great accuracy in distinguishing healthy subjects from diseased ones. βGLU activity stimulated by nonopsonized *Staphylococcus aureus* showed strongest correlation with the intensity of periodontal parameters compared to opsonized zymosan and prodigiosan, while the highest enzyme activity was stimulated by opsonized prodigiosan.

The strength of association between salivary βGLU activity, periodontitis and type 2 diabetes mellitus has been examined in dentate patients with different stages of periodontal disease, diabetic patients and edentulous patients [89]. In all subjects, diabetic status contributed significantly to βGLU activity, while periodontal status had greater influence on enzyme activity. Higher enzyme activity was also found in nondiabetic dentate patients with periodontitis compared to edentulous controls. Overall, compared to IL-1β, βGLU activity level was more reliable as biomarker of disease severity for periodontitis than it was for the presence of diabetes. In a predating study [90], GCF βGLU activity also correlated strongly with PD, CAL and BOP regardless of diabetic status. Lower enzyme activity was seen in diabetic subjects compared to those with periodontitis. The results suggested a lower release of βGLU in response to systemic inflammation (diabetes) due to reduced deficiency in neutrophil activity, in contrast to amplified activity in response to local inflammation (periodontitis).

2.2. Cancer

Despite landmark developments in oncology, the high rates of morbidity and mortality and increased medical costs associated with all forms of cancer coupled with patients' psychological trauma on disease diagnosis has remained a major threat to global public health. The successful disease management and sustained wellbeing of affected individuals is however subject to early disease diagnosis and constant evaluation of administered therapy. This in turn relies on efficient tumour markers to ascertain disease risk or status *i.e.* early stage or metastatic cancer [91]. Although a vast number of biomarkers have been identified for cancer diagnosis, only few have gained clinical approval due to inconsistencies and false positives in their utility [92]. A successful biomarker is that which will not only be specific and selective but will also predict treatment response, while differentiating lethargic and aggressive tumours.

The aetiology of cancer is known to be closely associated with inflammatory pathways and oxidative stress, which jointly create microenvironments favouring neoplasia [93,94]. Hence, in the tumour milieu, an increase in extracellular activity of lysosomal exoglycosidases responsible for the catalytic degradation of glycoconjugates occur, due to malignancy-mediated cell-death and/or lysosomal damage. In this vein, available practical data supports the overexpression of βGLU in extracellular fluids and tissues around tumour sites as a prime factor in cancer aetiology [24]; thus, suggesting the enzyme's viability as cancer biomarker [95].

For example, βGLU activity was 2-folds higher in the blood samples of 21 patients with colorectal adenocarcinoma compared to healthy controls [96]. Based on cell maturity and clinical grading, enzyme activity was highest in subjects with low or moderately differentiated cells and in subjects with tumours infiltrating surrounding tissues and organs or visceral peritoneum respectively. Estimation of serum βGLU activity in this case proved to be 80% sensitive and 82% specific in distinguishing diseased and healthy subjects. Likewise, enzyme activity was elevated by 6-fold in the peritoneal fluids of patients with ovarian or endometrial cancer compared to women with infertility used as controls [97]. The stronger correlation between cancer stage and βGLU activity, compared to the correlations of other lysosomal exoglycosidases *i.e.* β-galactosidase and α-mannosidase, reiterated the improved clinical viability of βGLU activity as biomarker of gynaecologic tumour status and severity. However, βGLU activity was equally elevated in the peritoneal fluid of patients with pelvic inflammation hence compromising its application for differential diagnosis of gynaecologic cancer and pelvic inflammatory disease.

2.3. Bacterial inflammation

Bacterial peritonitis is a life-threatening inflammation of the peritoneum – the tissues lining of the inner abdominal walls. As with other inflammatory conditions, cellular damage and

polymorphonuclear leukocytes activity increases the level of lysosomal enzymes in the extracellular space *via* enzyme leakage through the cellular membrane. Thus, quantification of these enzymes provides a potential diagnostic platform.

In fact, βGLU activity in the peritoneal fluid of patients with culture positive bacterial peritonitis was 9 and 33-fold greater compared to that of patients with acute mesenteric lymphadenitis and controls respectively [98]. Peritoneal fluid-βGLU activity measurement in this case holds greater clinical potential compared to β-galactosidase and α-mannosidase for early disease diagnosis and evaluating patient's response to treatment.

Similarly, considering the current global prevalence of antimicrobial resistance, timely diagnosis of bacteria-mediated meningeal inflammation (bacterial meningitis) is crucial to the outcome of therapeutic intervention. It has been shown that βGLU activity is elevated in cell-free cerebrospinal fluid (CSF) of bacterial meningitis patients early in the disease pathogenesis, even when traditional laboratory parameters such as number of CSF cells, CSF-blood glucose ratio and protein concentration indicated normal status [99]. CSF-βGLU as biomarker was superior to these traditional parameters for early and sensitive prediction of patient's response to antibiotic treatment.

CSF-βGLU activity in neonates and infants has also been studied as biomarker for differential diagnosis of sterile CSF pleocytosis due to urinary tract infection (UTI) or meningitis [100]. Median βGLU activity in CSF of patients showed significant difference without overlapping in each disease state *i.e.*, bacterial meningitis (168), viral meningitis (26.5) and UTI with sterile CSF pleocytosis (44.1); median enzyme activity was lowest (19.1) in febrile subjects without CSF pleocytosis used as controls. This proffers an unambiguous diagnosis of each condition with 100% sensitivity and specificity. In contrast, broad overlapping was found with classic CSF laboratory parameters *viz*., CSF cell number, neutrophil number, protein concentration and CSF-blood glucose ratio.

Recently, a case-control study concluded that βGLU activity in bronchoalveolar lavage fluid (BALF) is clinically useful as biomarker of bacterial lung infection [101]. In BALF samples from 92 children, enzyme activity was significantly higher in patients with positive BALF bacterial culture (C+) compared to those with culture negative BALF (C−). 43 nmol 4-methylumbelliferone (4MU)/ml/h was identified as optimum activity value, which allowed differential sample screening (*i.e.* C+ from C−) with 84.8% sensitivity and 78.3% specificity. Moreover, receiver operating characteristics (ROC) curve analysis established the superiority and higher prognostic value of βGLU activity for bacterial lung infection compared to % polymorphonuclear cell count, human leukocyte elastase, IL-8 and TNF-α.

2.4. Organophosphorus pesticide poisoning

Repeated exposure to organophosphorus compounds (OP) in pesticides is responsible for the lethal poisonings seen in agricultural and veterinary workers; particularly, those in developing countries. OP exerts their fatal effects by inhibiting acetylcholinesterase (AChE) in nervous tissues, leading to muscarinic and nicotinic effects with central nervous disturbance [102]. On the other hand, βGLU is retained in liver microsomal endoplasmic reticulum by forming non-covalent binding complexes with its accessory 64 kda glycoprotein, egasyn, a carboxylesterase isozyme [103,104]. Liver intake of OP however cleaves microsomal βGLU-egasyn complex thus elevating the level of βGLU in plasma to consequently making it an alternative biomarker for OP pesticide poisoning diagnosis [105–107].

A cross-sectional study of pest control workers [108] and plastic greenhouse workers [109], established that plasma βGLU activity was more sensitive as biomarker of OP poisoning compared to butyrylcholinesterase (BuChE) and acid phosphatase (AcP). The enzyme's activity was higher in subjects with increased exposure than those with low exposure and controls. βGLU activity correlated significantly with BuChE and AcP activities. Likewise, in a case-control study, serum βGLU activity was significantly increased in patients with severe poisoning compared to mildly affected patients and controls [110]. The difference in enzyme activity between the latter groups was insignificant.

Nonetheless, mildly exposed subjects have been reported to show elevated βGLU activity than severely exposed and control subjects, which suggests the enzyme's utility for diagnosing low-levels of OP exposure. For instance, a study [111], observed the order of serum βGLU activity based on poisoning severity as mild > severe > moderate poisoning, while the order after 12 and 24 h of admission was mild > severe ≈ moderate poisoning; strong correlation also persisted between serum βGLU and BuChE activities. Similar results were also obtained in a recent cross-sectional study [112]. Therein, moderately exposed subjects showed higher enzyme activity than the highly exposed, while non-significant statistical difference persisted between control and highly exposed groups. Notably, βGLU activity correlated well with diabetes propensity, lipid profile, liver function and BuChE but not AChE.

In another cross-sectional study, significant difference in plasma βGLU activity only exists between controls and subjects with chronic exposure (1–45 years) to OP [113]. Activity level was similar in controls and patients with acute poisoning; although 3 out of the 5 examined acutely poisoned patients showed increased level of plasma βGLU activity. However, a case report of an acute OP self-poisoned patient reached contrasting conclusion [114]. The opposing result can be linked to sample size and limited data on patient's medical history. Moreover, the reduced susceptibility of βGLU-egasyn complex to OP in humans is another primal factor [115]. In contrast to murine βGLU-egasyn interaction, binding in humans is independent of the *C*-terminal 18 amino acids propeptide in βGLU and esterase active site of egasyn. Rather, it involves the 51 amino residues in βGLU internal segment *i.e.* residues 228 to 279.

3. Inhibitors of β-glucuronidase activity

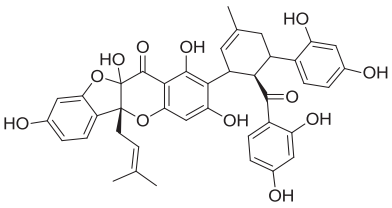
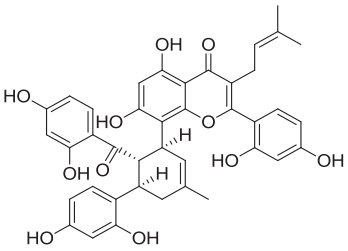
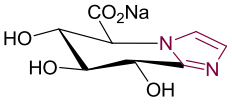
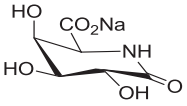
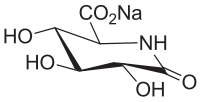
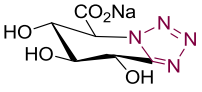
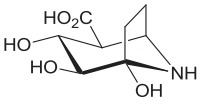
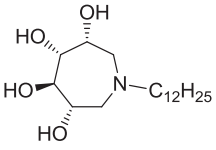
3.1. Natural products derived βGLU inhibitors

The progress and momentous achievements in drug discovery cannot be isolated from the wealth of chemical entities *i.e.* natural products, gifted to man by nature. Many successful molecular candidates of pharmaceutical drug discovery programmes are indebted to the presence of natural product-derived/inspired fragments in their scaffolds [116]. Therefore, the chemical space of natural products (both plants and microbes) has continued to be a much-researched depository for therapeutically significant molecules. In this section, we review some application of natural products including isolated compounds, whole plant extracts and natural product-inspired molecules as potent inhibitors of βGLU. The structures of selected inhibitors ($IC_{50} \leq 5$ μM) are presented in Table 2 at the end of the section.

3.1.1. Natural acids and lactones

The clarification [117,118] that D-saccharic acid-1,4-lactone (**5**, Fig. 3) (saccharolactone or D-glucaric acid-1,4-lactone) is the active and non-toxic βGLU inhibitor responsible for the strong inhibitory potency found with saccharate solutions has precipitated an increased interest in the compound. Despite the poor stability at physiological pH, D-saccharic acid-1,4-lactone (D-SAL) has been

Table 2
Most active natural product-derived βGLU inhibitors with $IC_{50} \leq 5$ μM.

Class	Compound	Structure	IC_{50} μM	Ref.
Flavonoids	**20**		*Eco*GUS = 0.40 *Spas*GUS = 0.33	[136]
	21		*Eco*GUS = 1.60 *Spas*GUS = 0.98	
Thiosulfinate	**52**		3.60	[165]
Iminosugars	**60**		12 nM[a]	[175]
	61		31 nM[a]	[174]
	62		32 nM[a]	
	63		25 nM[a]	
	77		60 nM[a,b]	[179]
	87		3.30[c]	[182]

[a] Inhibition constant K_i.
[b] Potency against *E. coli* βGLU.
[c] Absolute selectivity for *E. coli* βGLU.

explored extensively for its therapeutic significance. Although an IC_{50} value of 3.6 μM was reported in the pioneering work [117], a value *ca.* 40 μM is recurrent in literature. Nevertheless, at a concentration of 1 mM, D-SAL completely inhibited the hydrolytic action of human liver-derived βGLU on quercetin glucuronides [119], while over 90% inhibition of βGLU in breast milk was recorded at 10 μM [120]. Also, using urine samples of male Sprague-Dawley rats, D-SAL was identified *via* metabolomics strategy as one of the therapeutic constituents in LiuWeiDiHuang pills, a famous traditional Chinese prescription for cancer treatment and prevention [121]. Whereas, in a 6-days cumulative study, intraperitoneal pre or cotreatment of female Wistar rats with 3 mg/ml, 10 mg/ml or 10 mg/0.5 ml D-SAL reduced the severity of CPT-11 induced small-intestine mucosal damage assessed by the number of apoptotic cells or mitotic figures, compared to CPT-11 treated controls [122]. Damage reduction was independent of treatment schedule.

The synthetic and natural precursors of D-SAL *i.e.*, 2,5-di-*O*-acetyl-D-glucaro-1,4:6,3-dilactone (D-SDL, **6**, Fig. 3) and D-glucurono-γ-lactone (D-GL, **7**) respectively, have also potently inhibited βGLU activity in male Fischer rats thereby providing chemopreventive effects against azoxymethane-induced colon carcinogenesis [123]. Diet supplementation with 0.5 or 2% D-SDL for 5 weeks significantly reduced aberrant crypt foci formation (*i.e.* preneoplastic lesions) by over 48.6 and 55.3% respectively, compared

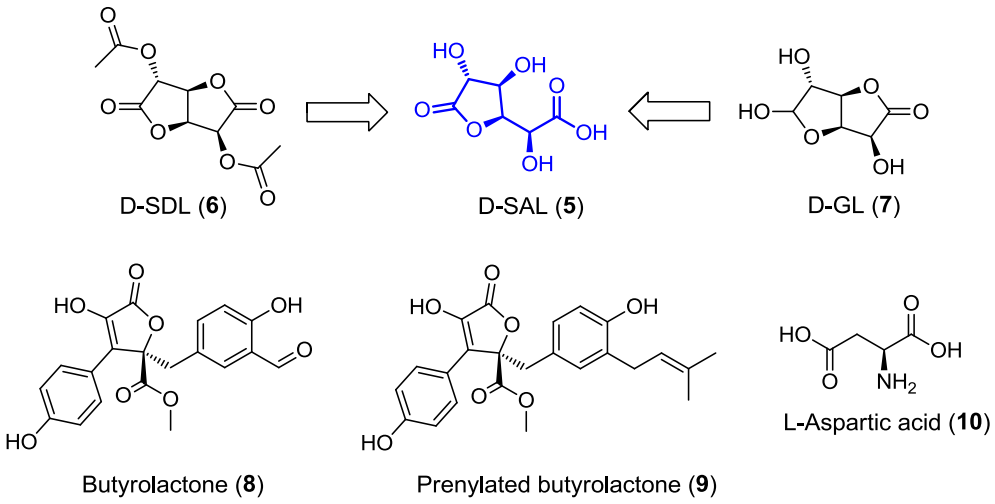


Fig. 3. Lactones and L-aspartic acid based βGLU inhibitors.

to azoxymethane-controls. D-GL did not afford any significant reduction in colonic tumour incidence during this initiation phase. In addition, 32 weeks treatment with D-SDL or high dose (2%) of D-GL, after 3-weeks subcutaneous injections of 15 mg/kg azoxymethane, provided over 70% inhibition of colon carcinogenesis during the post-initiation phase. It was suggested that D-SDL is a blocking agent which may inhibit pre-neoplasia during the initiation phase, while D-GL is only active during post-initiation of colon carcinogenesis. The increased hydrolytic stability of D-SDL to D-SAL *in vivo* might also be responsible for these observed phenomena [124].

In another study, D-SAL was more therapeutically efficient compared to its natural precursor (D-GL), for reducing epidermal hyperplasia (lethargic tumour promoter) and inflammation in 7,12-dimethylbenz(α)anthracene (DMBA)-induced complete skin carcinogenesis of SENCAR mice [125]. Pre and cotreatment of murine models with D-SAL for 4-weeks (twice weekly) by topical administration (0.5–4 mg) or dietary treatment (0.5 and 1%), both significantly reduced epidermal hyperplasia and inflammation by up to 57% of DMBA-controls in a dose-dependent manner. D-SAL also inhibited the initiation of carcinogenesis by reducing DMBA-induced oxidative DNA damage (C-8 hydroxylation of guanine) and mutations in codon 61 of Ha-*ras* gene, by up to 78%. In contrast, D-GL only inhibited epidermal hyperplasia with topical treatment and inflammation by dietary treatment (5% in diet); albeit with inferior potency compared to D-SAL.

Interestingly, another lactone-based βGLU inhibitor (**8**, Fig. 3), exhibited 8-fold superior potency compared to D-SAL [126]. Isolated from the ethyl acetate extracts of *Aspergillus terreus*, an endophytic fungus initially isolated from marine alga *Laurencia ceylanica*, butyrolactone (**8**) with IC_{50} = 6.2 μM possesses 16-fold stronger potency than its prenylated isomer (**9**).

βGLU in breast milk is believed to be involved in neonatal jaundice and hyperbilirubinemia, by increasing serum bilirubin levels *via* enterohepatic bilirubin circulation in breastfed newborns, in contrast to those fed with infant formulas [47]. Therefore, the suppression of βGLU-mediated deglucuronidation has been proffered as a practicable regimen. L-aspartic acid (**10**, Fig. 3) in casein hydrolysate formulas was identified as the active βGLU inhibitor responsible for the lower levels of neonatal jaundice observed in newborns receiving such formulae [127]. At 100 μM, the natural amino acid showed ~86% inhibition of βGLU that is, 100-fold more potent than D-isomer. Moreover, in a randomized and double-blind clinical trial involving 64-newborns, supplementing breastfeeding in the first week of life with 6 doses of L-aspartic acid (180 mg/5 ml of water/day) was more potent against βGLU activity than higher concentrations of enzymatically hydrolysed casein (infant formula containing the inhibitor) or whey/casein (routine formula lacking the inhibitor) [128]. L-aspartic acid supplementation at minimal aliquot concentration significantly lowered transcutaneous bilirubin levels (25% lower than control), leading to higher faecal bilirubin excretion and reducing neonatal jaundice, with no adverse effects.

3.1.2. Flavonoids

Flavonoids are evidently an indispensable class of natural products due to their ubiquity in the vegetal domain and their therapeutic significance. Found in a variety of plant parts (leaves, flowers, stems, nuts, seeds *etc.*), they perform important functions especially plant's growth and protection against pathogenic invasion. This intrinsic property encourages their utility as a major constituent of different local medicinal formulations and diets, while their acceptable toxicity profile and physiologic tolerance further presents them as druggable subjects. Flavonoids are typified by the C6–C3–C6 ring system that is, their basic structural skeleton consists of two benzene rings (A and B) linked by heterocyclic pyran ring C (Fig. 4). Variations on the chromane core (ring A and C) and attachment position of ring B due to biosynthetic origins lead to classification into flavones, flavanols, flavanones, flavonols, isoflavones, neoflavonoids, anthocyanins and chalcones [129]. This ring system and the presence of hydroxyl units has availed flavonoids with different pharmacological properties such as antidiabetic, antioxidant, antimicrobial, antiplasmodial, antiproliferative and particularly enzyme inhibition [130,131].

Luteolin (**11**, Fig. 4), a dietary 3′, 4′, 5, 7-tetrahydroxyflavone has been reported as a viable chemopreventive and anticarcinogenic agent plausibly by inhibiting bacterial βGLU-mediated enterohepatic circulation of colonic carcinogens [132]. The 30-weeks cumulative study using male Wister rats showed that pre or co-treatment with luteolin by intragastric gavage *per os* (*p.o.*) at 0.1, 0.2 or 0.3 mg/kg body weight/day, significantly reduced bacterial βGLU activity thereby suppressing 1,2-dimethylhydrazine (DMH)-induced colon adenocarcinomas in a dose-dependent manner compared to DMH-controls. Although 0.2 and 0.3 mg kg^{-1} day^{-1} produced similar result. Luteolin supplementation also reduced tumour size from 2 cm to 0.25 and 0.50 cm during the initiation and

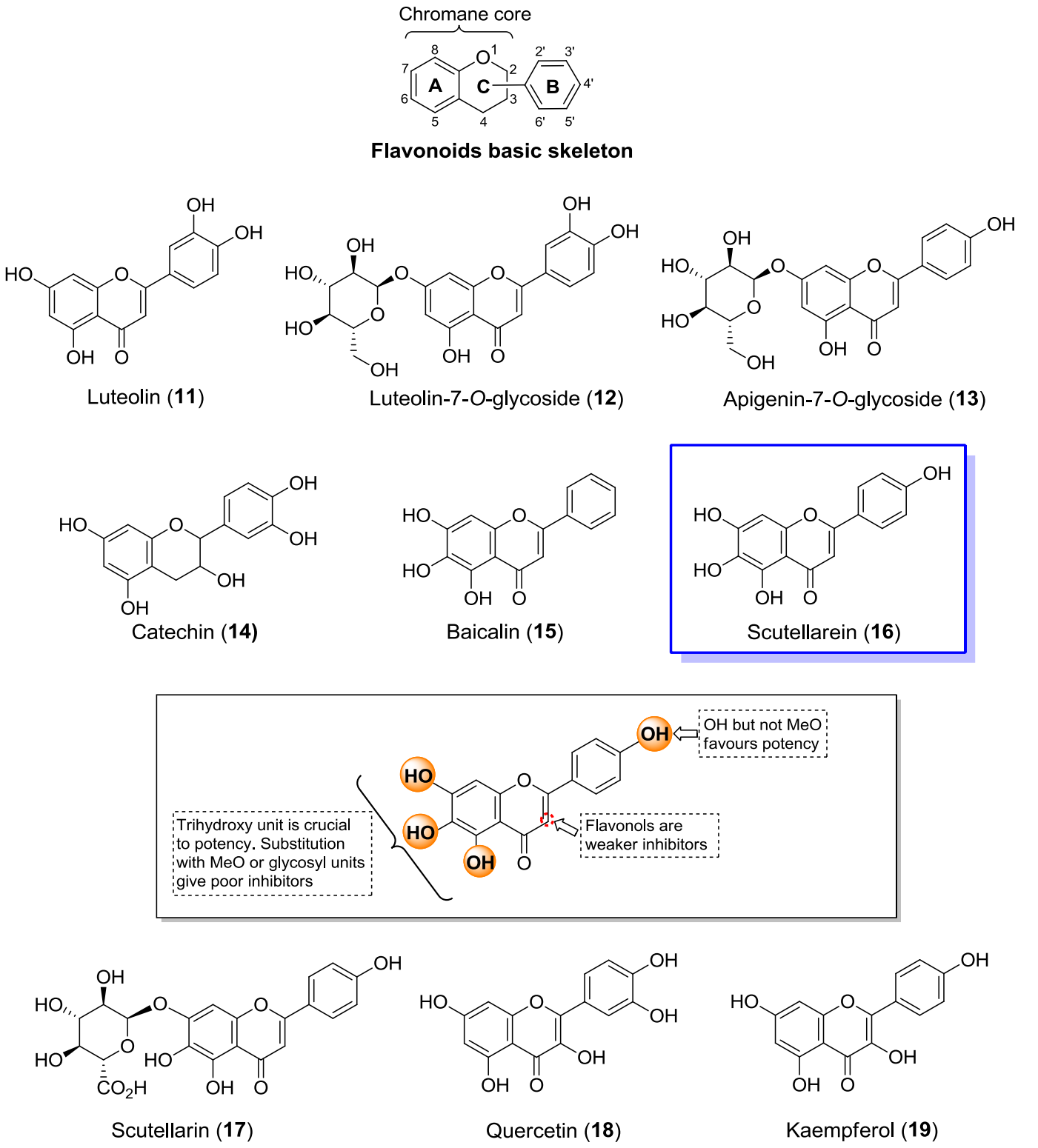


Fig. 4. Natural flavonoids (most active in blue box) and SAR analysis for *E. coli* βGLU inhibition. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

post-initiation stages respectively, as well up to 90% reduction in tumour incidence. In addition, luteolin (**11**), its 7-*O*-glycoside (**12**), apigenin-7-*O*-glycoside (**13**) and catechin (**14**) were identified as key constituents in the leaf extracts of *Pistacia terebinthus* responsible for the high *E. coli* βGLU inhibitory potency [133]. At the highest test concentration (8.2 μg/ml), the leaf extracts exhibited 97.2% inhibition of βGLU activity, corresponding to an IC_{50} value of 2.11 μg/ml.

In another exploit, 32 natural flavonoids were evaluated for their inhibitory strengths against *E. coli* βGLU [134]. It was

established that luteolin (**11**), similar flavones – baicalein (**15**), scutellarein (**16**) and its glucuronidated analogue scutellarin (**17**), as well as dietary and ubiquitously occurring flavonol – quercetin (**18**), are superior inhibitors (IC_{50} = 5.76–29.64 μM) compared to reference inhibitor D-SAL (IC_{50} = 36.07 μM); isoflavones and dihydroflavones displayed weaker inhibition. Overall, luteolin (**11**) and scutellarein (**16**) emerged the most potent flavone-based *E. coli* βGLU inhibitors with IC_{50} = 8.68 and 5.76 μM respectively. SAR analysis (Fig. 4) revealed the importance of 5,6,7-trihydroxy (pyrogallol) unit to bacterial βGLU inhibition, as unsubstitution or replacement of a hydroxy unit with methoxy or glycosyl unit led to significant loss of inhibitory activity. *O*-methylation at positions-6, 7 and 4′ and installing OH unit at position-3 was also detrimental to potency, whereas the presence of hydroxy unit at C-4' favoured potency. In addition, molecular docking studies of luteolin and scutellarein in the active site of *E. coli* βGLU showed hydrogen bonding (H-b) interactions of phenolic OH units at C-5 and C-7 with catalytic acid Glu413 and Arg562 as well as hydrophobic contacts with Ser360 and Leu361 in the bacterial loop.

Furthermore, subjecting the methanolic extracts of edible flower and pedicel of aquatic rhizomatous herb – *Nymphaea pubescens* (water lily) to bioactivity-guided fractionation established the superior (3-fold) bacterial βGLU inhibitory potency of crude flower extracts compared to pedicel extracts and silymarin – a marketed natural βGLU inhibitor [135]. Kaempferol (**19**) was subsequently identified as one of the active metabolites with promising activity; IC_{50} = 36.47 μM and 76-fold superior to silymarin. Interestingly, the lower activity of kaempferol (**19**) compared to flavonoids (**11**, **15**–**18**) affirmed the SAR results described earlier.

Amidst 21 constituents found in commonly used Chinese herbal medicines, two prenylflavonoids, Sanggenon C (**20**, Fig. 5) and Kuwanon G (**21**) emerged as the most potent broad-spectrum inhibitors (>70% inhibition; IC_{50} = 12.5 and 7.4 μM respectively) of whole human gut bacterial βGLU (consisting of eight bacterial isolates), compared to reference compound amoxapine (7.3% inhibition) [136]. Compound **20** exhibited improved potency against recombinant βGLU from *E. coli* (*Eco*GUS) and *S. pasteuri* (*Spas*GUS), IC_{50} = 0.40 and 0.33 μM respectively, compared to compound **21** (IC_{50} = 1.6 and 0.98 μM respectively). However, compound **21** was a stronger inhibitor of βGLU from three representative bacterial isolates (*E. coli*, *E. fergusonni* and *S. pasteuri*) compared to compound **20**. Additionally, molecular docking studies of **20** and **21** in the binding pockets of *Eco*GUS and *Spas*GUS revealed both flavonoids bind (*via* their hydroxy groups) in the allosteric site and not the active site of the recombinant enzymes. The higher number of H-b interactions formed by compound **21** supported the overall increased potency compared to compound **20**.

The realization that pro-inflammatory mediators such as βGLU, released from degranulated mast cells or neutrophils, play significant roles in inflammatory disorders has sparked increased interest in the search for potent inhibitors of such processes as anti-inflammatory drug candidates. In this regard, dihydroxychalcones **22** and **23** (Fig. 6) isolated from the roots of *Hypericum geminiflorum* holds therapeutic promise [137]. Compounds **22** and **23** potently inhibited the release of βGLU from degranulated rat neutrophils with IC_{50} values of 5.80 and 6.60 μM respectively, better than the reference inhibitor trifluoperazine. However, only compound **23** showed moderate activity against the enzyme's release from degranulated rat peritoneal mast cells with IC_{50} = 70.0 μM. Conversely, an isomer of luteolin – norartocarpetin **24** (Fig. 6) and flavonoid-like mornigrol D **25** (Fig. 6), both isolated from the barks of *Morus nigra* (black mulberry), were moderately potent βGLU inhibitors [138]. At 100 μM, compounds **24** and **25** showed 67.7% and 65.9% inhibition respectively against the release of βGLU from activated rat neutrophils. However, chiral flavonoid alkaloids isolated from the ethanolic extracts of *Scutellaria moniliorrhiza* and subsequently separated using chiral HPLC, – scumonilines **26**–**29** (Fig. 6), are better drug candidates compared to compounds **24** and **25**, but similar to compounds **22** and **23** [139]. Scumonilines **26**–**29** displayed ~63% inhibition at 10 μM against βGLU release from activated rat neutrophils with IC_{50} values of 5.21, 5.85, 5.47 and 5.16 μM respectively; better than reference inhibitor ginkgolide B (IC_{50} = 6.63 μM). Compounds **26**–**29** were also more potent lead molecules compared to both glucuronate esters **30**–**34** (Fig. 6) isolated from similar *Scutellaria regeliana* [140] and chiral egenine-based alkaloids **35**–**37** isolated from the rhizomes of *Corydalis decumbens* [141]. Compounds **30**–**34** showed 43.7–47.1% inhibition at 10 μM, whereas compounds **35**–**37** were more inferior inhibitors at the same concentration with 32.4–41.3% inhibition.

3.1.3. Plant extracts and ethnomedicinal preparations

Evidently due to its phycocyanin-rich content and potent reduction of zymosan-induced damage to knee joint histological architecture, edible blue-green microalgae – spirulina is deemed a therapeutically viable anti-arthritic agent [142]. An analysis of the synovial fluid of female OF1 mice knee joints after 4-days intra-articular injection of zymosan, followed by 8-days oral administration of spirulina water-suspension at 100 mg/kg and 400 mg/kg, showed 78.7% and 89.2% inhibition of βGLU activity respectively. Subsequently, arthritic parameters such as tibial articular cartilage destruction, erosion of bone structure, articular tissue inflammation, loss of general joint architecture and pannus formation, were all markedly reduced in spirulina fed mice compared to those receiving zymosan only. These anti-inflammatory and anti-arthritic effects were comparable to reference drug Triamcinolone, a

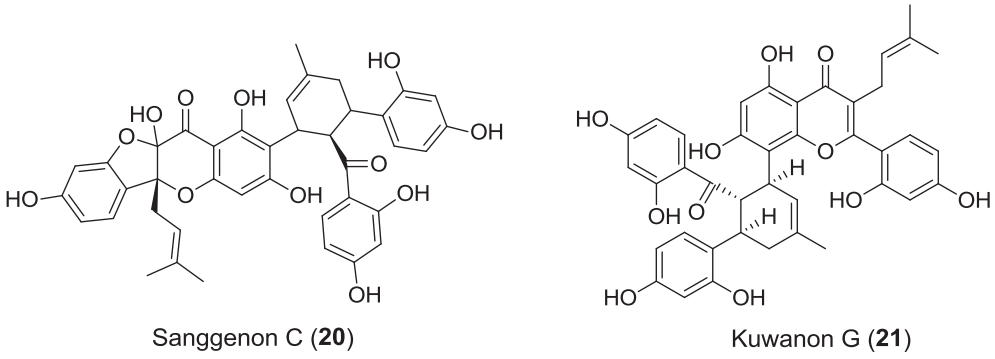


Fig. 5. Potent prenylflavonoids for bacterial βGLU inhibition.

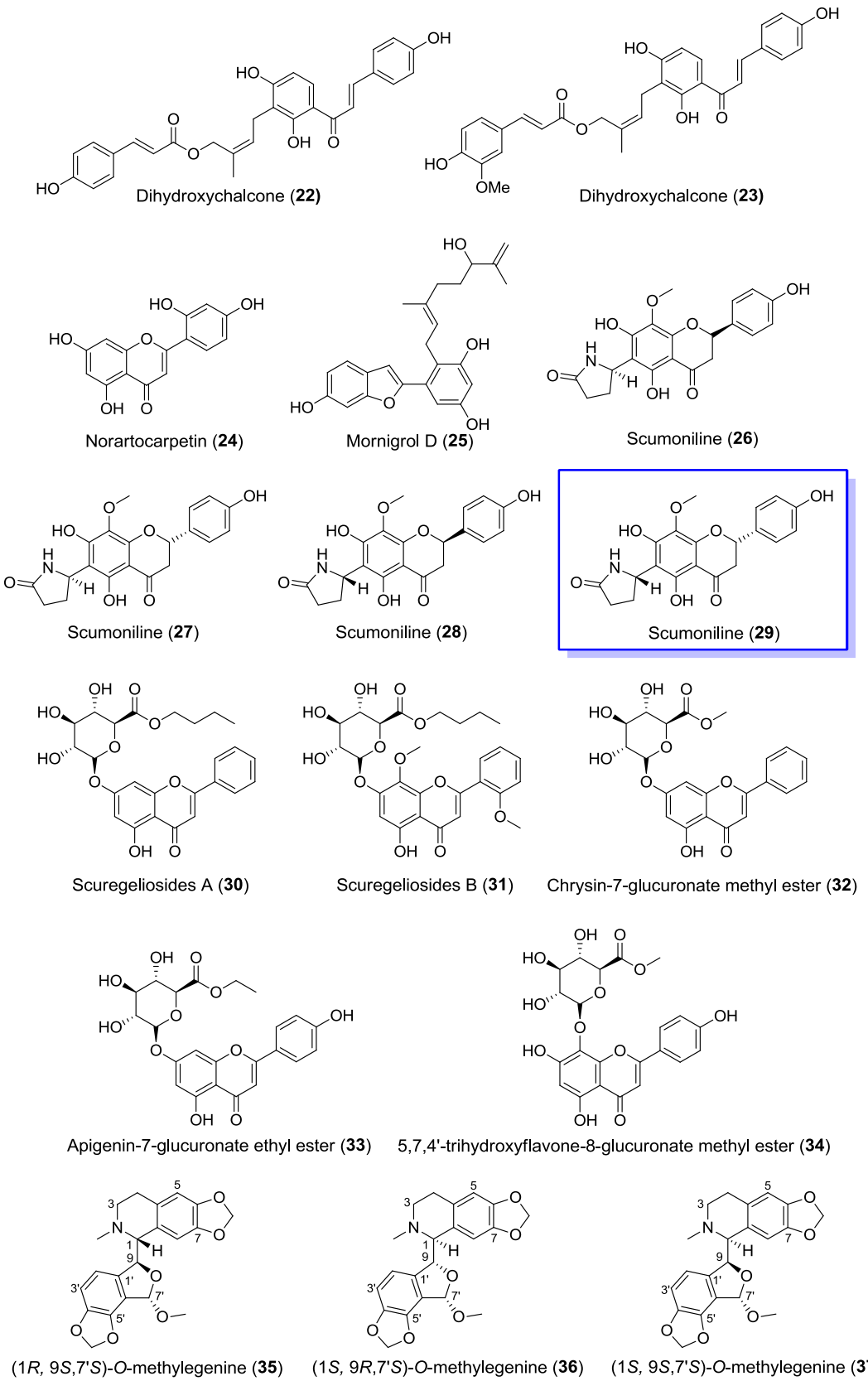


Fig. 6. Reported natural inhibitors of βGLU release from activated neutrophils and most potent chiral flavonoid alkaloid (in blue box). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

glucocorticoid with 94.1% βGLU inhibition at 10 mg/kg of body weight.

Tuber extracts of *Arisaema tortuosum* (Whipcord Cobra Lily) has shown moderate anti-inflammatory effect *via* βGLU inhibition in a dose-dependent manner [143]. Conceivably, the presence of quercetin, rutin and lectin, identified through chromatographic profiling, facilitated maximum inhibition of 92.9% at 100 mg/ml, superior to reference inhibitor salicylic acid. Employing a similar approach with ginger rhizomes [144], only 6-Gingerol (**38**, Fig. 7) was identified with 85% inhibition of βGLU at 1 mM, comparable to salicylic acid (82% inhibition). It is also noteworthy that constituents profiling of essential oils extracted from the leaves of seven local varieties of *Piper betle* L. identified eugenol (**39**) with an IC_{50} value of 616.68 μg/ml similar to 794.62 μg/ml of silymarin; although the essential oils were only moderate βGLU inhibitors with $IC_{50} \geq 5.26$ mg/ml [145]. However, metabolomics profiling of the leafy shoots-methanolic extracts of three *Swertia* species *viz. S. chirayita, S. decussata* and *S. bimaculate*, presented *S. chirayita* as the strongest inhibitor of βGLU and xanthones as the most active metabolites responsible for the *Swertia* species' potency [146]. The C-2-glycoside of norathyriol, mangiferin (**40**) emerged as the most active and therapeutically promising xanthone with $IC_{50} = 38$ μM and 50-fold more potent than silymarin.

Shikunshito-Kamiho (SKTK), a traditional oriental formulation and Fenugreek seeds (FGS), an Indian spice, are natural ethnotherapeutic agents with potentials identical to luteolin (**11**) *i.e.*, attenuation of colon carcinogenesis *via* potent inhibition of bacterial βGLU activity [147,148]. 5-weeks oral administration of SPF male ICR mice's diet supplemented with 20 mg/kg or 60 mg/kg SKTK water extracts, after 10 weeks subcutaneous injection of DMH (weekly), equipotently reduced βGLU activity during and after treatment by 14.9% and 21.3% of controls (DMH alone) respectively. Tumour incidence in colon, measured by the number of aberrant crypt foci was also significantly reduced from 21.6 (distributed in sigmoid and descending colons) in controls, to 6.9 and 6.4 (distributed in rectum) at 20 mg/kg and 60 mg/kg doses respectively. Similarly, diet supplementation with FGS significantly reduced intestinal βGLU activity, leading to a marked reduction in tumour incidence from 93.3% in male Wister rats receiving DMH alone for 15 weeks to 16.6% in rats receiving DMH + 2 g/kg FGS water extracts for 30-weeks cumulative period. Flavonoid, saponin and fibre rich content of FGS were presumed to be responsible for its anticarcinogenic activity.

The hepatoprotective activity of Chinese medicinal formulation – Reduohanxiao-tang, used in the treatment of stroke and liver diseases has also been attributed to its ability to inhibit bacterial βGLU activity *in vivo* [149]. Water extracts of this local formulation and two of its ingredients *viz*. rhizomes of *Pueraria thunbergiana* and *Scutellaria baicalensis* potently inhibited the activities of *E. coli* and rat liver-derived βGLU with IC_{50} values ranging from 0.35 to 1.42 mg/ml. Subsequently, oral administration of these extracts at 100 mg/kg alleviated CCl_4-induced liver injury measured by significant reduction in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactic acid dehydrogenase (LDH) levels, compared to controls. The superior hepatoprotective effect of *Pueraria thunbergiana* was accredited to isoflavone daidzein (**41**, Fig. 7), an aglycone metabolite of main components puerarin and daidzin by intestinal bacteria. Daidzein inhibited *E. coli* and rat liver βGLU with $IC_{50} = 0.41$ and 0.50 mg/ml respectively whereas puerarin and daidzin were inactive.

Fascinatingly, structurally similar tectorigenin (**42**), an aglycone metabolite of 7-*O*-glycoside – tectoridin (**43**), isolated from the flowers of *Pueraria thunbergiana*, exhibited better inhibitory potency ($IC_{50} = 0.30$ mg/ml) than its congeners [150]. 50 mg/kg intraperitoneal pre-treatment of male ICR mice with tectorigenin or 100 mg/kg oral administration of tectoridin provided better hepatoprotection than dimethyl diphenyl bicarboxylate (DDB) and daidzein, by significantly lowering serum AST, ALT and LDH levels relative to CCl_4-treated controls. The prodrug behaviour of tectoridin, identical to puerarin and daidzin above, was also established by its absence in the serum after 250 mg/kg oral administration (only tectorigenin was detected) and its failure to provide desired hepatoprotection after 100 mg/kg intraperitoneal administration.

3.1.4. Terpenoids and steroids

The triterpenoid 18β-glycyrrhetinic acid (**44**, Fig. 8) also known as enoxolone, is the aglycone metabolite by human intestinal bacteria responsible for the hepatoprotective activity exhibited by saponin – glycyrrhizinic acid **45** [151]. Glycyrrhizinic acid (**45**) is isolated from the rhizomes of *Glycyrrhiza uralensis* – the sweetening agent in SKTK. It exists as a natural glucuronide conjugate of 18β-glycyrrhetinic acid (**44**) hence making it (**45**) a substrate for βGLU-mediated hydrolysis for consequent release of active inhibitor **44**. *In vitro* evaluation against *E. coli* and rat liver βGLU revealed stronger potency of glycyrrhizinic acid ($IC_{50} = 12.15$ and 97.21 μM respectively) compared to 18β-glycyrrhetinic acid ($IC_{50} = 509.90$

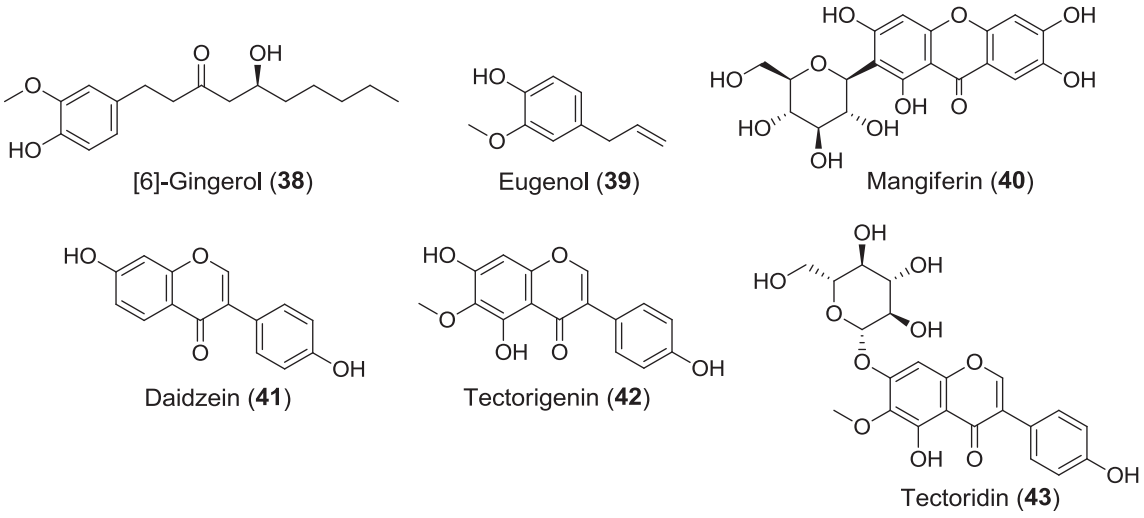


Fig. 7. Natural βGLU inhibitors profiled from plant extracts and ethnomedicinal preparations.

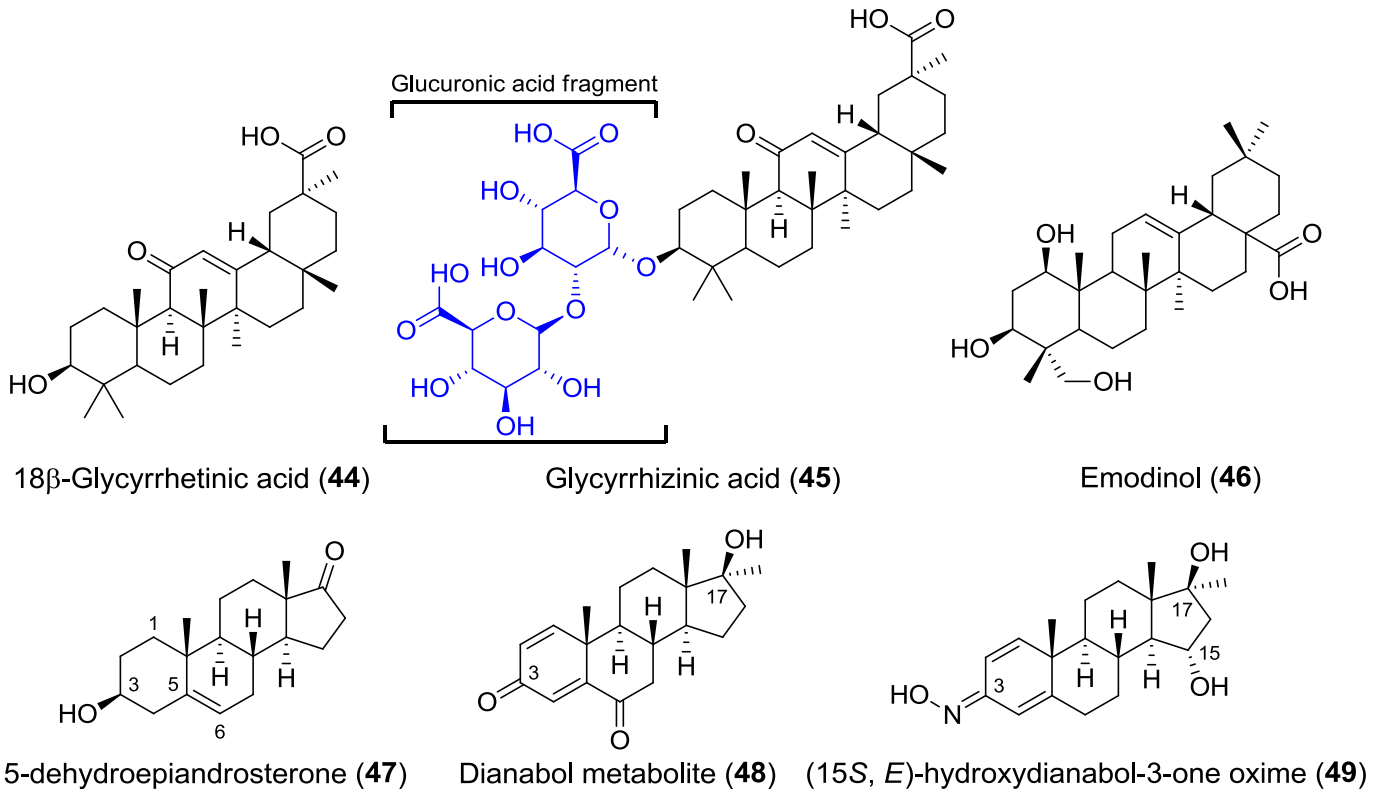


Fig. 8. Triterpenoids and steroids with promising bacterial βGLU inhibitory potency.

and 42.49 μM respectively). Moreover, at 100 mg/kg doses, oral treatment with glycyrrhizinic acid or intraperitoneal treatment with 18β-glycyrrhetinic acid, protected the hepatocytes of male Wistar rats against CCl_4-induced liver injury evident by the significant reduction in AST, ALT and LDH levels compared to CCl_4-controls. Intraperitoneal administration of glycyrrhizinic acid also did not provide hepatoprotection akin to tectoridin (**43**). In addition, structurally similar emodinol (**46**), isolated from the roots of perennial herb *Paeonia Emodi* showed stronger *E. coli* βGLU inhibition *in vitro* with IC_{50} = 63 μM compared to 18β-glycyrrhetinic acid [152].

Microbial biotransformation of drugs and/or their precursors to new molecules with modulated pharmacological profile is considered an invaluable tool in drug design and development [153]. The natural product class – steroids, are key substrates in this regard [154]. Accordingly, naturally occurring androstane steroid hormone, 5-dehydroepiandrosterone (**47**, Fig. 8) and its *Macrophomina phaseolina* metabolites were examined for their βGLU inhibitory potentials [155]. The inferior potency/inactivity of metabolites compared to parent 5-dehydroepiandrosterone (IC_{50} = 77.9 μM) suggests that having an alkene unit at position-5 and cyclopentanone unit in the molecular architecture is crucial to inhibitory potency. Conversely, the synthetic anabolic steroid, dianabol and its metabolites by filamentous fungus *Cunninghamella elegans* or *Macrophomina phaseolina* were inactive against βGLU; except metabolite (**48**) with IC_{50} = 60.7 μM [156]. The *E*-oxime derivative (**49**) of an inactive metabolite however showed decent inhibitory potency with IC_{50} = 49.0 μM, equipotent with D-SAL and 2-fold superior than its *Z*-isomer. Nonetheless, the therapeutic safety of these dianabol derived βGLU inhibitors is questionable considering the increased risk of hepatotoxicity associated with oral administration of 17α-alkylated anabolic steroids [157,158].

3.1.5. Lactic acid bacteria

The fascinating ability of prebiotics and probiotics to positively influence the composition and behaviour of human intestinal microbiota, has been continuously explored with considerable success to improve human health [159–161]. Precisely, lactic acid bacteria (LAB) probiotics can selectively utilize non-digestible oligosaccharide prebiotics as carbon source to produce active metabolites, which modulates or stimulates the activities of certain intestinal bacteria hence, eliciting important physiological response and conferring health benefit [162].

Consequently, LAB – *Lactobacillus acidophilus* CSG afforded stronger inhibition of intestinal bacteria (including *E. coli*) producing βGLU thus providing hepatoprotection to male ICR mice, compared to *Lactobacillus brevis* HY7401 and *Bifidobacterium longum* HY8001 [163]. When anaerobically cocultured with *E. coli* (HGU-3), *L. acidophilus* CSG also potently inhibited βGLU productivity of *E. coli* compared to other LABs. As a result, oral treatment of the murine models with 500 mg/kg of *L. acidophilus* CSG alleviated CCl_4-induced hepatotoxicity by lowering AST and ALT levels to 66% and 57% respectively, of CCl_4 control group. Whereas, for *t*-BHP-induced hepatotoxicity, AST and ALT levels were lowered to 62% and 48% respectively, better than reference hepatoprotective agent DDB. Similarly, *Lactobacillus plantarum* CFR 2194 was the most effective strain of *Lactobacilli* metabolizing fructooligosaccharide prebiotics to short chain fatty acids, thereby altering βGLU productivity of *E. coli* [164]. Lactic acid (**50**, Fig. 9) and especially n-butyric acid (**51**) were identified as the major short chain fatty acid metabolites in the culture filtrate of *Lactobacillus plantarum* CFR 2194 and fructooligosaccharides responsible for the observed decrease in βGLU activity.

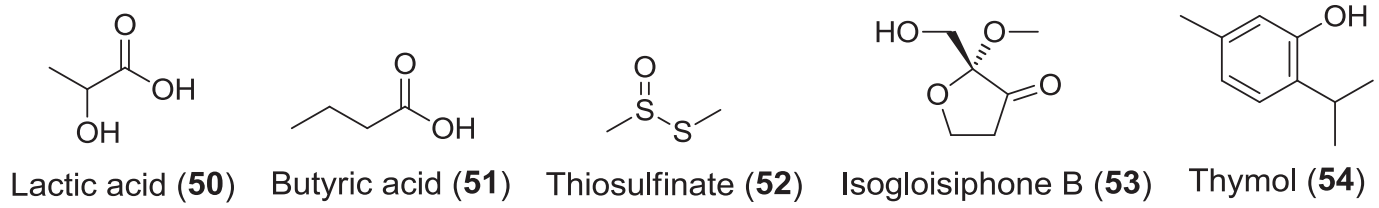


Fig. 9. Simple molecules with potential chemopreventive effects *via* βGLU inhibition.

3.1.6. Other plant isolates

The most abundant and relatively stable thiosulfinate, *S*-methyl methanethiosulfinate (**52**, Fig. 9), found in Chinese chive (*Allium tuberosum* Rottier), has exhibited strong inhibitory potency (IC_{50} = 3.60 μM) against *E. coli* βGLU [165]. Compared to other disulphides *viz*., dimethyl, allyl methyl, and diallyl disulphides, compound **52** is more useful for alleviating drug induced toxicities or providing hepatoprotection. In parallel, a naturally occurring acetal isolated from red macroalga *Neodilsea yendoana*, isogloiosiphone B (**53**), possesses similar potential, although with weaker potency (IC_{50} = 57.0 μM) compared to compound **52** [166].

The adverse role of inflammatory pathways and corresponding mediators including lysosomal enzymes during the pathogenesis of myocardial infarction again implicates βGLU in the cardiovascular disease. In a study which examined the protective effects of thymol (**54**, Fig. 9) on inflammation in isoproterenol-induced myocardial infarction using male albino Wistar rats [167], the therapeutic benefit of inhibiting the release of βGLU, other lysosomal enzymes and pro-inflammatory cytokines was evident in the restoration of near-normal myocardial histology and function, compared to diseased controls. Rats pre and cotreated with thymol by intragastric gavage at 7.5 mg/kg body weight/day for 7 days and subcutaneous injection of 100 mg/kg isoproterenol for 2 days (day 6 and 7), showed significantly reduced levels of cardiac troponin-T (a cardiotoxicity biomarker) and high-sensitive C-reactive protein in their sera, compared to those treated with isoproterenol only. Most importantly, the activities of lysosomal enzymes *i.e.* βGLU, β-galactosidase, cathepsin B and D in serum and heart were also potently inhibited to near-normal. These anti-inflammatory effects of thymol afforded a well-protected myocardium with stable histological architecture.

3.1.7. Iminosugars

Polyhydroxylated piperidine and pyrrolidine alkaloids commonly referred to as iminosugars (Fig. 10a), are a noble class of sugar mimics in which the pyrano or furano endocyclic oxygen has been replaced with a basic nitrogen atom [168]. This class also include isoiminosugars (*i.e.* 1-azacarbasugars or 1-*N*-iminosugars) having a methylene unit in place of endocyclic oxygen and the anomeric carbon replaced with nitrogen. Although these ring alterations render iminosugars metabolically inert, their protonated forms mimic the pyranosyl or furanosyl unit in GH substrates, especially the putative oxocarbenium ion-like transition state (**2**, Fig. 2) in GH catalysed hydrolysis [21,169]. This in turn facilitates their recognizability by GHs and other carbohydrate-recognizing proteins for corresponding enzyme inhibition. Since the isolation of nojirimycin (**55**, first iminosugar) and siastatin B (**56**, first isoiminosugar) from *Streptomyces* cultures in 1966 and 1974 respectively [170,171], these sugar mimics have been a recurring subject of intensive research owing to their extensive biological activities and therapeutic applications elicited majorly through potent inhibition of GHs [172]. The strongest iminosugar-based inhibitors of bovine liver-derived βGLU reported so far (Fig. 10b), with promising anti-tumor potentials are siastatin B derivatives **57**, **58** and **59** with IC_{50} = 65, 62 and 68 nM respectively [173]. Therefore, this section reviews iminosugars and their analogues as natural product-inspired βGLU inhibitors, without undue repetitions of other monographs [4,21,168,169] covering them.

Uronic analogues of nojirimycin bearing glycaro-1,5-lactams or the bicyclic analogues with imidazole and tetrazole units (*i.e.* tetrahydrotetrazolopyridine-5-carboxylates and imidazopyridine-5-carboxylates respectively), were prepared to examine the effect of sugar-acid configuration and presence of lactam, tetrazole or imidazole moieties on inhibitory potency against bovine liver βGLU [174,175]. Evidenced by the most potent inhibitor **60** (Fig. 11) with K_i = 12 nM, *gluco*-configured units, are stronger βGLU inhibitors compared to *galacto*- and *manno*-analogues, due to their better mimicry of glucuronic acid (**4**). The imidazole ring also conferred stronger inhibitory potency than tetrazole or lactam units whereas glycarolactams and tetrazoles shared similar potencies; except *galacto*-tetrazole (**64**) with ~200-fold inferior potency to *galacto*-lactam (**62**). Further, although sugar configuration at C-4 was found to be ineffective on βGLU inhibition for glycaro-1,5-lactams **61** and **62**, it was very significant for tetrazole and imidazole derivatives. *Gluco*-tetrazole (**63**) was 300-fold better than *galacto*-tetrazole (**64**), while *gluco*-imidazole (**60**) was 600-fold more potent than *galacto*-imidazole (**65**) and 1200 superior to *manno*-imidazopyridine (**66**), which differs at C-2. Moreover, sugar configuration of acid moiety at C-5 also had significant influence on potency. L-configured units **67**, **68** and **69** were 20-50-fold weaker than their D-isomers **63**, **62** and **61** respectively. More importantly, the 2-fold increased potency of *gluco*-imidazole (**60**) compared to *gluco*-tetrazole (**63**) was attributed to stronger interaction of *gluco*-imidazole (**60**) with catalytic nucleophile Glu540 compared to *gluco*-tetrazole (**63**). Interaction with catalytic acid Glu451 was compromised due to protonation of the imidazole ring in the zwitterionic form.

Conversely, similar bicyclic molecules of nojirimycin with cyclic carbamate, urea or guanidine pharmacophores are weaker βGLU inhibitors [176]. In all, only cyclic carbamates **70**–**73** (Fig. 12) showed moderate inhibitory activity against bovine liver βGLU. Cyclic carbamates with carboxylic acid unit at C-5 of nojirimycin ring were also better inhibitors than their hydroxymethyl analogues. Thus, compounds **71** and **73** were the most potent overall with IC_{50} = 218 and 259 μM respectively. It is noteworthy that bicyclic indolizidine iminosugar **74**, with hydroxymethyl unit is also a weak inhibitor (<50% inhibition at 1 mM), although it exhibits desirable selectivity (7-fold) for *E. coli* βGLU [177]. Surprisingly, carbasugar **75** shared a similar fate with IC_{50} = 170 μM against *E. coli* βGLU, even though it is not an iminosugar [178]. Taken together, these results partly suggest that ability to mimic D-glucuronic acid favours βGLU inhibitory activity.

Accordingly, glucuronic acid analogue of naturally occurring hemiaminal calystegine B_2 (**76**, Fig. 13), uronic-noeurostegine (**77**), was synthesized in 27-steps from levoglucosan [179]. Compound **77** strongly inhibited both bovine liver and *E. coli* βGLU with K_i values of 2.3 and 0.060 μM respectively; *i.e.* 38-fold selectivity for the bacterial ortholog. The serendipitously obtained *N*-alkylated

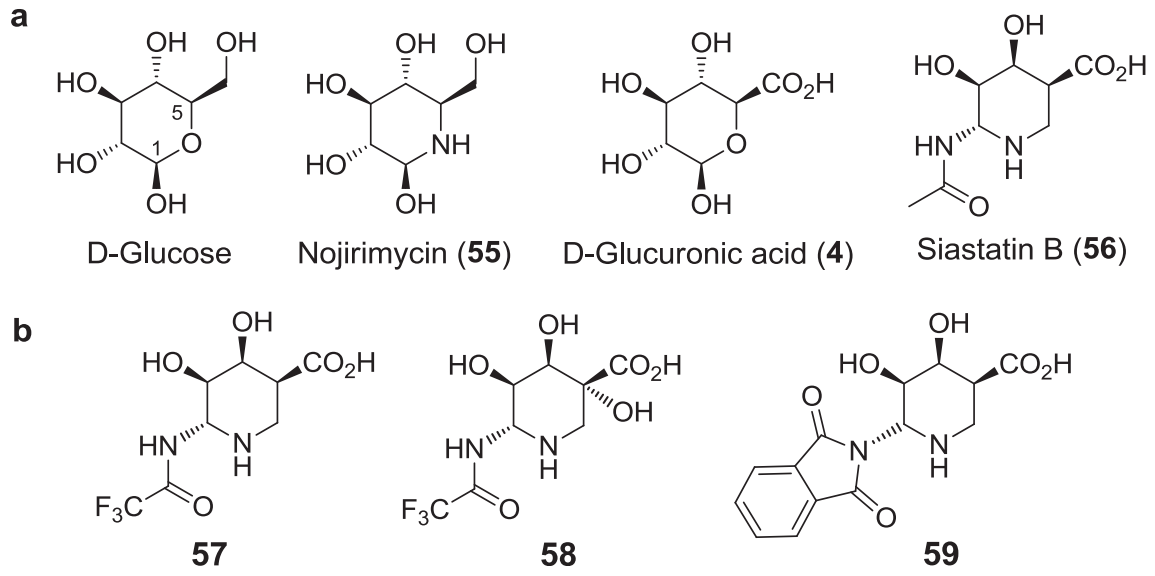
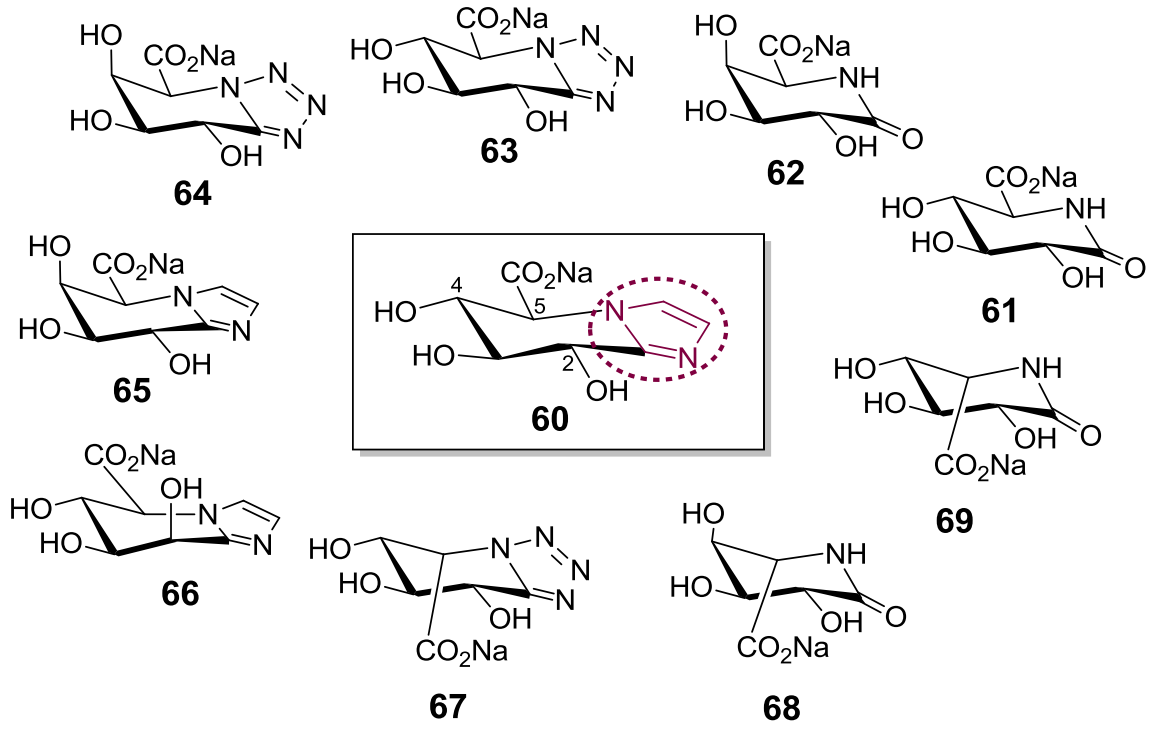


Fig. 10. Iminosugars. (**a**) Nojirimycin and siastatin B, sugar mimics of D-glucose and D-glucuronic acid (**b**) Most potent siastatin B-inspired βGLU inhibitors with antitumor potentials.



Compound	**60**	61	62	63	64	65	66	67	68	69
K_i (μM)	**0.012**	0.032	0.031	0.025	6.3	6.7	6.6	0.66	0.60	1.6

Fig. 11. Uronic analogues of nojirimycin containing lactam, tetrazole and imidazole units with most potent βGLU inhibitor, compound **60**.

derivatives of compound **77** *viz*., *N*-4-hydroxylbutyl (**78**) and *N*-ethyl (**79**), also showed 5 and 10-fold selectivity respectively for *E. coli* βGLU, although with weaker potencies compared to parent compound **77**. Interestingly, *uronic*-noeurostegines **77**–**79** were selective inhibitors of βGLU as the compounds were inactive at 1 mM against other GHs examined. Molecular docking studies of **77** revealed that it binds in the central catalytic pocket of *E. coli* βGLU, forming H-b interactions *via* its NH and 2-OH units with catalytic acid Glu413 and catalytic nucleophile Glu504 in the bacterial loop. The 4-OH unit of **77** also formed H-b interaction with side chain carboxyl unit of Asp183, while the carboxyl unit at position-5 interacted similarly with phenolic OH of Tyr472, guanidine NH of Arg562, side chain NH_2 of Lys568 and amide NH_2 of Asn566.

Iminosugar *C*-glycosides are a different class of iminosugars

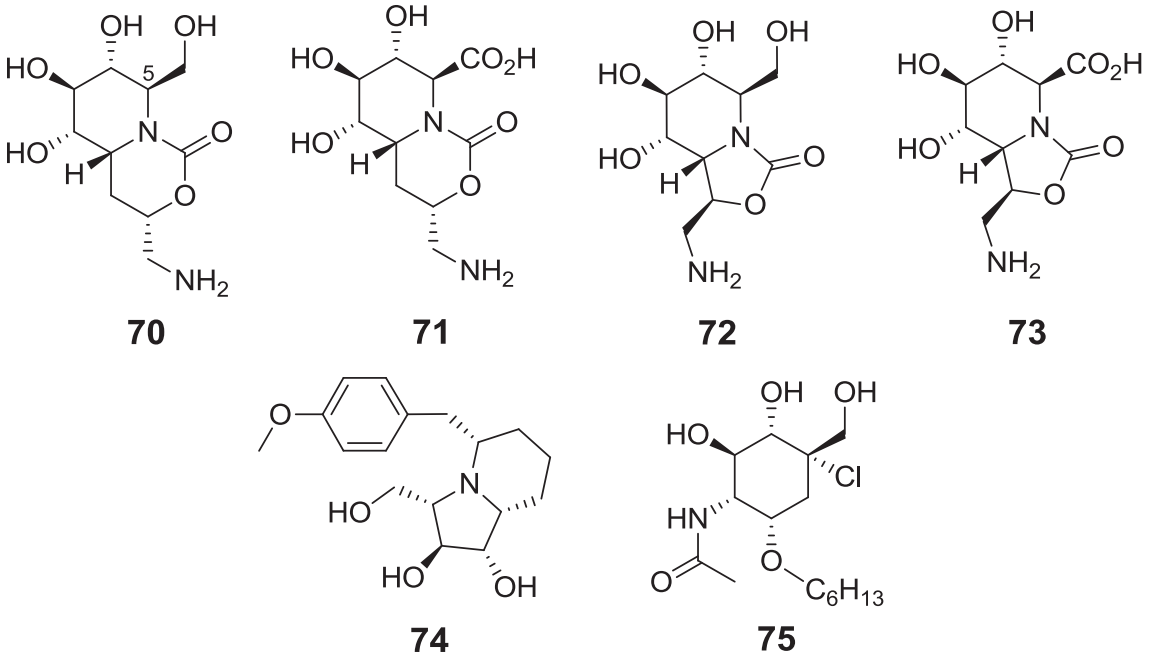


Fig. 12. Bicyclic nojirimycin-carbamates, indolizidine iminosugar and carbasugar-based *E. coli* βGLU inhibitors.

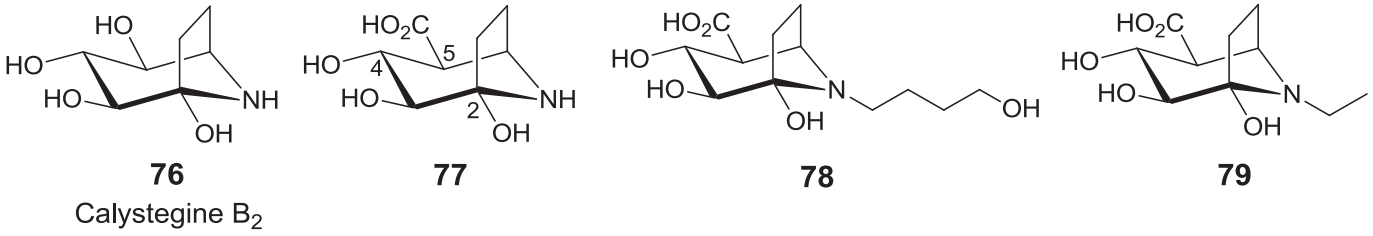


Fig. 13. Calystegine B_2-inspired uronic-noeurostegines as selective *E. coli* βGLU inhibitors.

conferred with improved selectivity for *E. coli* βGLU. This is partly due to their improved lipophilic balance for bacterial cell penetration afforded by their *C*-alkyl unit and improved chemical stability of C—C bond at the pseudoanomeric C-1 centre [180]. L-iminosugar *C*-glycoside, (−)-adenophorine (**80**, Fig. 14a), an enantiomer of natural iminosugar *C*-glycoside (+)-adenophorine (**81**), was prepared together with it analogues *via* skeletal rearrangement of corresponding azepanes [181]. However, compound **80** and analogues were found to be weak but selective inhibitors of *E. coli* βGLU. The *C*-propyl analogue, compound **82** with IC_{50} = 586 μM showed total selectivity for the bacterial ortholog. It was also superior to compound **80**, its azepane precursor **83**, and other iminosugars in the study which showed moderate selectivity with 3.1—18.1% inhibition at 1 mM.

However, increasing the lipophilicity of azepane scaffold **84** *via* *C*-2 or *N*-alkylation, while tuning the configuration of alkyl and hydroxy substituents at C-2 and C-6 respectively (Fig. 14b), birthed *E. coli* βGLU inhibitors with markedly increased potency and highly conserved selectivity [182]. SAR analysis revealed that a combination of (6*S*)—OH unit and *N*-alkylation with hexyl, nonyl or dodecyl produced stronger inhibitors and their potency increased with increasing chain length. In contrast, (2*R*, 6*R*)-*C*-butyl and (2*S*, 6*R*)-*C*-nonyl derivatives, compounds **85** and **86** respectively, were the most active *C*-alkylated analogues with IC_{50} = 261 and 27 μM respectively. Compound **85** was stronger than its *N*-alkylated analogue, while compound **86** showed a compromised selectivity (IC_{50} = 97 μM against bovine liver βGLU). Overall, highly lipophilic compound **87**, having (6*S*)—*N*-dodecyl framework was the strongest inhibitor with IC_{50} = 3.30 μM, 3-fold superior potency to (6*S*)—*N*-nonyl analogue **88** (IC_{50} = 10.0 μM) and absolute selectivity for *E. coli* βGLU. Furthermore, substituent type and configuration at C-6 also influenced the inhibitory potencies of noeuromycin azepane analogues [183]. (6*S*)-configured compound **89** and **90** were weakly potent, compared to their inactive (6*R*)-configured analogues. Compound **89** (IC_{50} = 139 μM) was 4-fold more potent than its 6-hydroxymethylated analogue compound **90**.

Incorporating the acetamido unit in Siastatin B (**56**), while retaining the iminosugar *C*-glycoside scaffold *via* similar skeletal rearrangement of azepanes, afforded L-configured *C*-glycosides of 1-deoxynojirimycin (**91**) with weak inhibitory potencies [184]. Sugar configuration at C-1 and C-2 (Fig. 14d), significantly influenced selectivity for GHs. Hence, (2*R*, 3*R*)-configured molecules were selective inhibitors of *E. coli* βGLU but inactive against bovine liver βGLU, α-*N*-acetylgalactosaminidase and β-*N*-acetylgalactosaminidase. Whereas, (2*S*, 3*S*)-configuration infused selectivity for β-*N*-acetylgalactosaminidase. Consequently, (2*R*, 3*R*)-configured compound **92** with a rigid benzyl substituent emerged as the best *E. coli* βGLU inhibitor in the series with IC_{50} = 90.7 μM compared to 37.2% inhibition at 1 mM of compound **93** with (2*S*, 3*S*)-configuration and butyl unit. The authors attributed these inferior activities to stereochemical mismatch between the L-configured units and D-configured substrates of GHs. We also conceive that the presence of

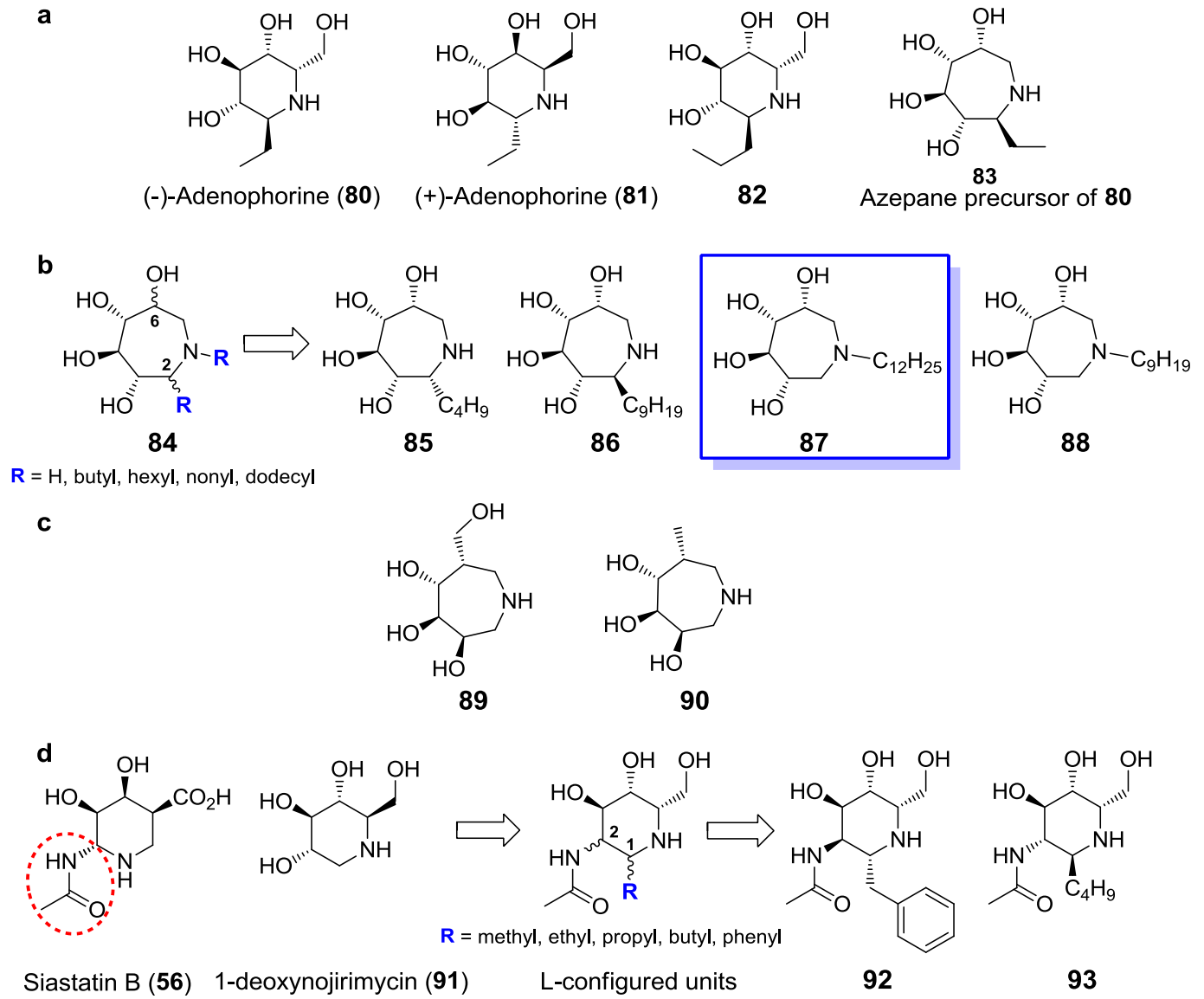


Fig. 14. Iminosugar *C*-glycosides with most potent *E.* coli βGLU inhibitor overall in blue box. (**a**) weakly potent (−)-adenophorine analogues (**b**) azepanes with markedly increased potency and high selectivity for *E.* coli βGLU (**c**) noeuromycin azepane analogues (**d**) L-configured iminosugar *C*-glycosides bearing acetamido unit. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

hydroxymethyl and not carboxylic unit (to mimic glucuronic acid) might also be responsible for the weak inhibitory potencies. This was indeed the case for 3,4,5-trihydroxypipecolic acids, uronic analogues of 1-deoxynojirimycin [185]. D-isomers were stronger than corresponding L-isomers, while the most active βGLU inhibitors were those with better mimicry of glucuronic acid *viz.* D-*gluco* and D-*galacto* configured units; compounds **94** and **95** respectively (Fig. 15). Compound **94** showed 3-fold selectivity for bovine liver βGLU (IC_{50} = 70 μM), whereas compound **95** displayed an exclusive inhibition (IC_{50} = 86 μM).

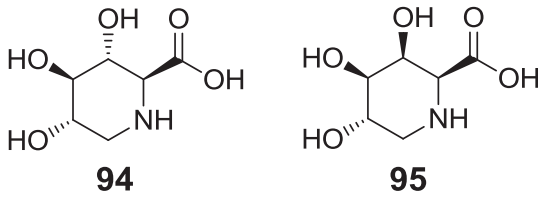


Fig. 15. Potent uronic-acid analogues of 1-deoxynojirimycin.

3.2. Synthetic βGLU inhibitors

3.2.1. Azoles

Azoles are a prominent class of heterocycles with at least one nitrogen atom in their 5-membered aromatic ring. Due to their structural rigidity and amazing physicochemical properties, which confers highly coveted and broad pharmacological activity spectrum and therapeutic potentials, they have remained a targeted scaffold of many synthetic protocols. Members of this class include pyrazoles, imidazoles, thiazoles, triazoles, oxadiazoles, thiadiazoles and tetrazoles together with their benzo analogues *i.e.*, indoles, benzimidazoles, benzothiazoles and benzotriazoles. These compounds also enjoy increased hydrogen bonding (H-b) capability furnished by ring *N* and/or *O* atoms for biomolecular targets binding. Consequently, this section is an overview of reported βGLU

inhibitors bearing one or more azole nuclei. The critical focus is on the most potent inhibitor(s) in a given series, the pharmacological profile, structure-activity relationship (SAR) and molecular docking analysis.

3.2.1.1. Imidazole. Metronidazole backbone has bestowed superior βGLU inhibitory potency (IC_{50} = 1.20–44.16 μM) on a set of imidazolylethylaryl carboxylates compared to reference inhibitor D-SAL (IC_{50} = 48.38 μM) [186]. Compound **96** (Fig. 16) emerged as the most potent βGLU inhibitor (IC_{50} = 1.20 μM) with 40-fold superior potency to D-SAL. SAR analysis supported by *in silico* studies articulated that compounds with electron-donating groups (EDGs) displayed inferior inhibitory activities compared to those with electron-withdrawing groups (EWGs). Moreover, molecular hybridization with indole nucleus resulted in a potent βGLU inhibitor compound **97** with IC_{50} = 2.10 μM and 23-fold increased potency than D-SAL. Compound **96** and **97** displayed strong H-b interaction of indole NH unit and π-π interaction with active site residues of βGLU.

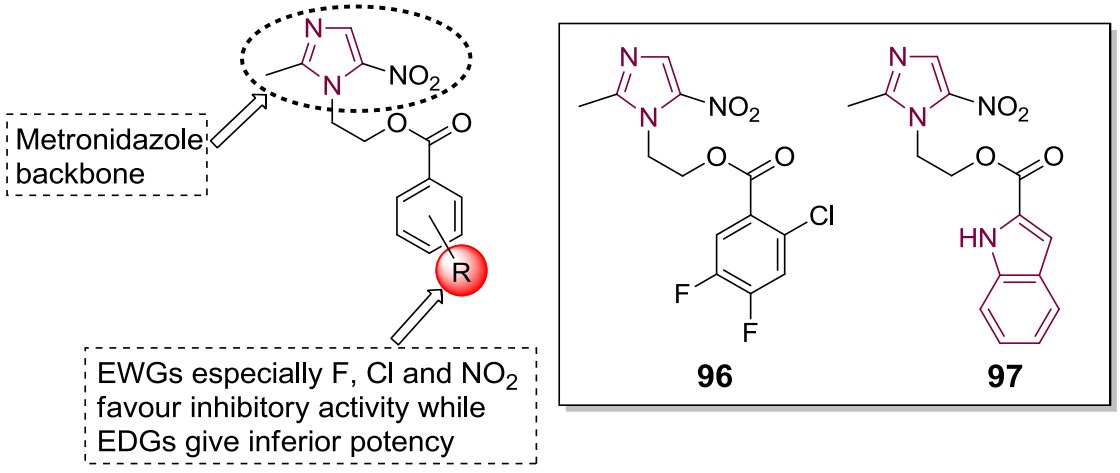
In vitro screening of imidazolopyridines (Fig. 17) for their βGLU inhibitory potentials presented dihydroxy-substituted derivatives as promising inhibitors of enzyme activity [187]. The most active molecules in the series are dihydroxy substituted compounds **98** and **99** with IC_{50} values of 29.25 and 30.10 μM respectively, *i.e.*, 2-fold improved potency compared to D-SAL. Docking simulations revealed the importance of adjacent OH groups to favourable binding interaction with βGLU. Position-2-OH in compound **98** interacted *via* H-b with both phenolic OH of Tyr504 and NH unit of Lys606, while position-3-OH interacted similarly with backbone carboxylate group of Asp207. Likewise, position-3-OH unit in compound **99** showed H-b interactions with Asn604 and Lys606, while its 4-OH unit interacted with Asp207. Conceivably, these adjacent hydroxyl groups influenced the favourable orientation of free NH unit for strong H-b interaction with catalytic acid Glu451; an interaction which was absent for weakly potent molecules such 2,4- and 2,5-dihydroxy derivatives.

In another study adopting similar imidazolopyridine skeleton [188], only compound **100** (Fig. 18) showed appreciable activity (IC_{50} = 33.01 μM), compared to D-SAL (IC_{50} = 45.75 μM). The presence of *para*-methyl substituted on phenyl ring at position-2 of imidazolopyridine ring favoured inhibitory activity plausibly by increasing hydrophobicity, as the unsubstituted derivative **101** had 2-fold decreased potency. However, uninstalling trifluoromethyl (CF_3) moiety at position-7 or replacing the furan ring at position-5 with Me, CF_3, phenyl or thiophene rendered the resulting molecules inactive.

3.2.1.2. Thiazole. SAR analysis of thiazole-Schiff bases showed the significance of OH, Cl and NO_2 substituents on benzylidene fragment to inhibitory potency against bovine liver βGLU [189]. 2,3-diOH and 2-OH-5-Cl substituted compounds **102** and **103** respectively, were the strongest inhibitors in the series with IC_{50} values of 4.88 and 5.63 μM respectively (Fig. 19). However, replacing OH unit with more lipophilic MeO or EtO groups led to significant loss of activity. Molecular docking analysis also disclosed the importance of iminic nitrogen and proximity of thiazole nitrogen to catalytic acid Glu451 for strong inhibitory potency. Compound **102** with adjacent OH units had a favourable fit into βGLU catalytic pocket for stronger interactions with amino acid residues Glu540, Glu451 and Tyr508.

However, installing pyren-1-ylmethylenehydrazinyl moiety on thiazole skeleton improved βGLU inhibitory potency [190]. Compounds **104** and **105** (Fig. 20) emerged as the most potent inhibitors with IC_{50} = 3.10 and 3.20 μM respectively. Interestingly, the thiosemicarbazone intermediate compound **106** was 9-fold stronger than D-SAL (IC_{50} = 48.38 μM), while 12-fold improved potency was observed for the thiazolone variant (**107**). Binding mode analysis of compounds **104** and **105** revealed hydrophobic contacts *via* pyrene units with Met556 and Phe557. Specifically, the hydrazine unit in compound **104** formed H-b with Arg600, arene-arene interaction with Tyr508 *via* its thiazole ring and arene-cation contact with Arg600 *via* the pyrene unit. *ortho*-OH unit in compound **105** also formed H-b interaction with Asp207, while both thiazole nitrogen and *ortho*-OH interacted with Arg600. These interactions were also observed in compounds **106** and **107** but absent in other molecules with inferior potencies.

3.2.1.3. 1,3,4-Thiadiazole. SAR analysis of 1,3,4-thiadiazole-based βGLU inhibitors disclosed the strong dependence of inhibitory potency on 3,4-diCl substituent of *N*-phenyl ring as compounds



R	3,4-diCl	2,5-diCl	2-Br-4-F	3-Me-4-NO_2	3-F	6-benzo thiazolyl	4-NO_2	4-F	3-Cl	2-Cl	4-Me
IC_{50} μM	3.60	6.20	6.30	7.30	8.10	8.40	8.90	12.40	16.30	19.30	24.30

Fig. 16. SAR of imidazolylethyl aryl carboxylates and most potent compounds **96** and **97**.

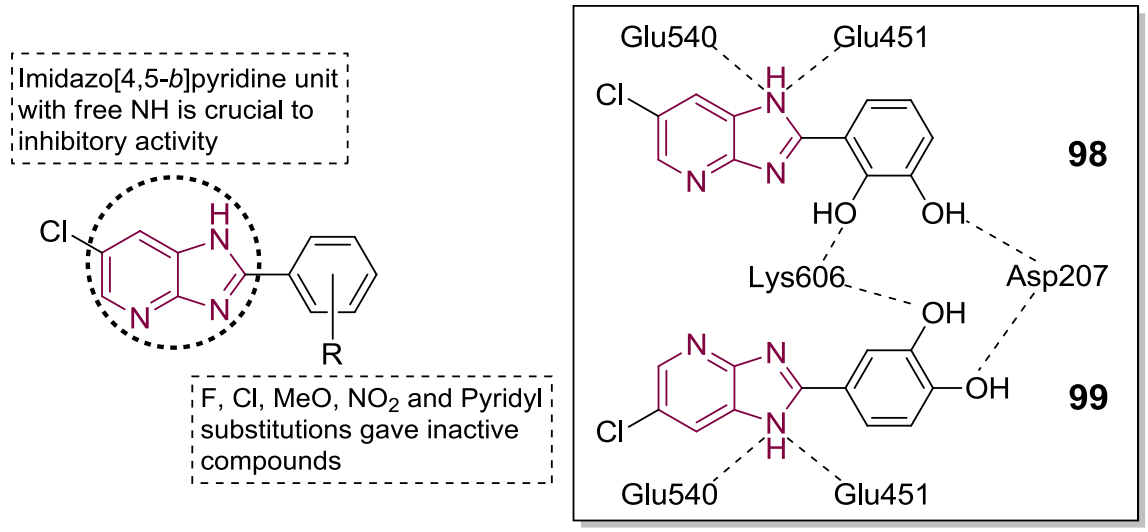


Fig. 17. Imidazo[4,5-*b*]pyridine analogues and binding modes of most active inhibitors **98** and **99**.

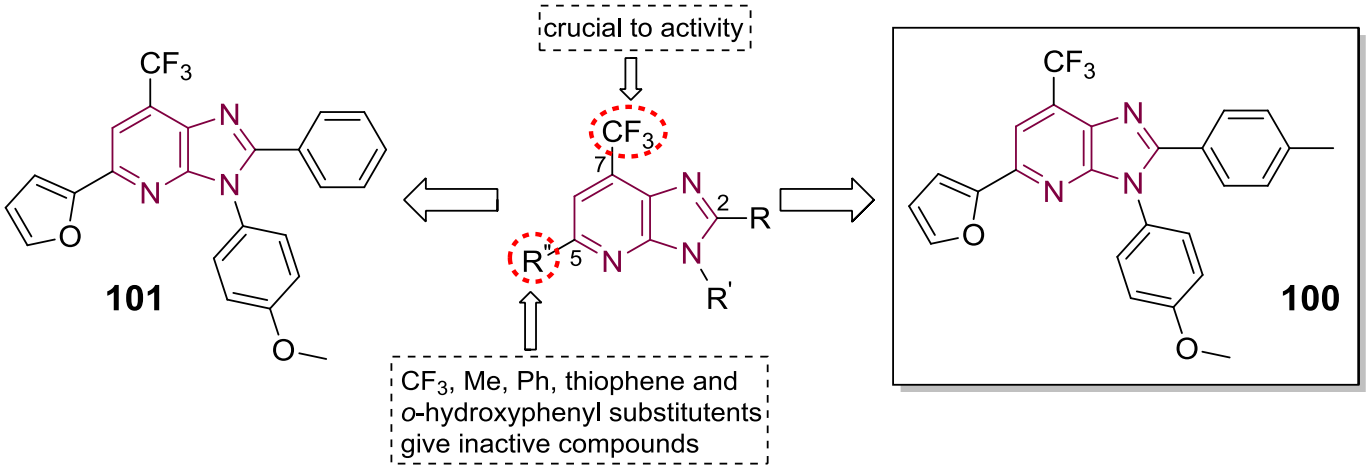


Fig. 18. 7-(trifluoromethyl)imidazo[4,5-*b*]pyridines and most active inhibitor **100**.

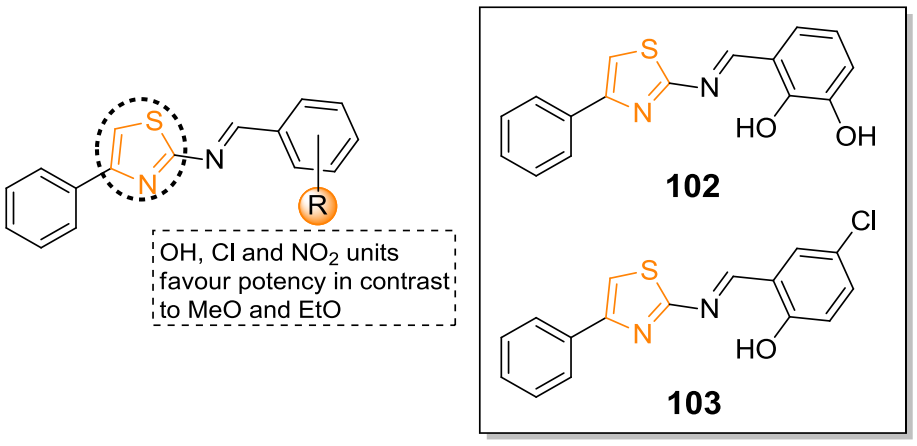
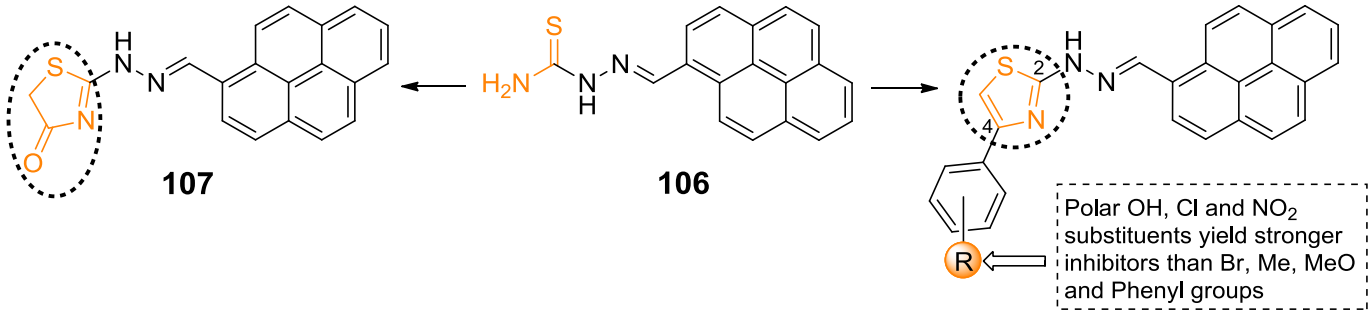
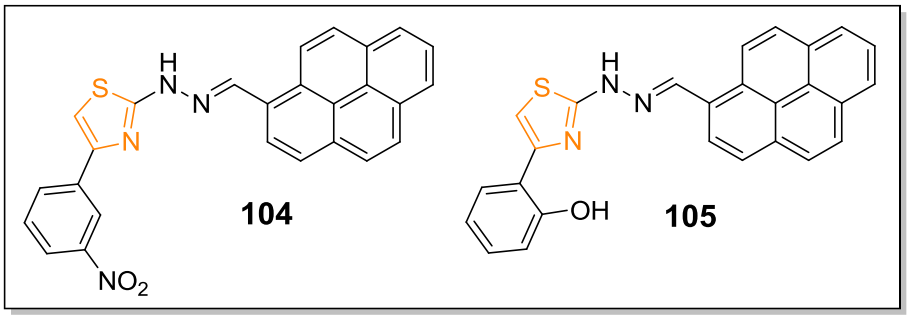


Fig. 19. Most active thiazole-Schiff base-derived βGLU inhibitors.

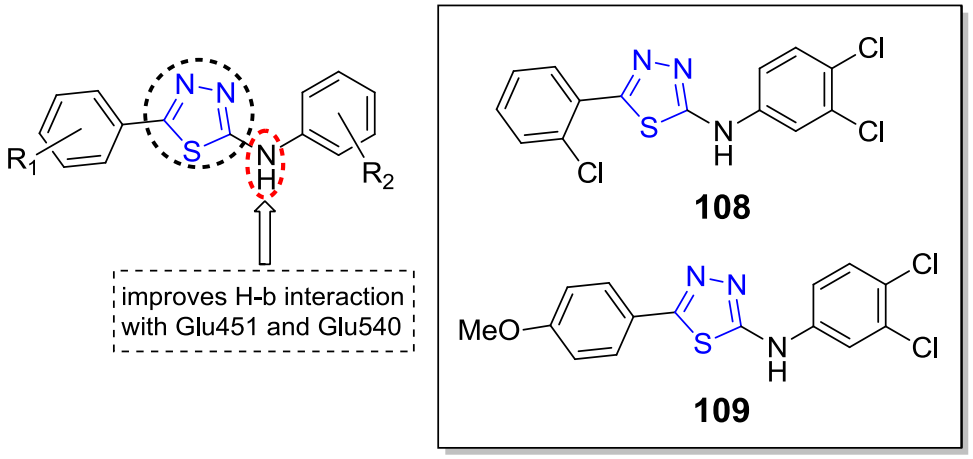
with Br, Me and MeO substituents were weaker inhibitors [191]. As a result, compound **108** with $IC_{50} = 2.16$ μM (Fig. 21), was the most potent inhibitor. It was 2 and 22-fold stronger than compound **109** and D-SAL respectively. Docking studies revealed that the free amino NH unit in compound **108** formed strong H-b interaction with OH unit of catalytic acid Glu451. The dichloro substituents also





R	3-NO_2	4--NO_2	2-OH	3-OH	3-Cl	4-Cl	3,4-diCl	3-Br	4-Br	4-Me	**106**	**107**
IC_{50} μM	3.10	25.60	3.20	8.50	23.60	11.10	6.20	35.40	31.60	24.40	**5.50**	**4.10**

Fig. 20. Thiazole-pyrenes and most active inhibitors **104** and **105**.



R_1	**2-Cl**	2-Cl	2-Cl	2-Cl	**4-MeO**	Phenyl	4-Br	4-Br	2,4-diMe	3,5-diMeO
R_2	**3,4-diCl**	3-Br	4-Me	4-MeO	**3,4-diCl**	3,4-diCl	3,4-diCl	4-MeO	3,4-diCl	3,4-diCl
IC_{50} μM	**2.16**	8.96	16.65	22.61	**4.16**	6.28	7.16	31.06	15.61	19.16

Fig. 21. Thiadiazole derived βGLU inhibitors and most potent compounds.

aided hydrophobic interactions with Tyr504 and Tyr508, while both thiadiazole and phenyl rings interacted *via* π-π stacking with Asp207. On the hand, 2-fold reduced potency of compound **109** was revealed by its poorer binding modes in the enzyme's active site.

3.2.1.4. Benzimidazole-based hybrids. The inhibitory potency of benzimidazole nucleus against βGLU was investigated using substituted phenyl units at position-2 of benzimidazole core [192]. Activity was dependent on the presence and position of Cl and OH substituents hence 3,4-dichlorophenyl analogue **110** (Fig. 22) was found with strongest inhibitory potency (IC_{50} = 6.33 μM). Interestingly, introducing 5,7-dichloro unit on benzimidazole nucleus gave remarkable results as previously inactive molecules gained significant activity [193]. Compound **111** emerged as the most potent inhibitor overall with IC_{50} = 4.48 μM and 11-fold stronger than D-SAL. Moreover, replacing an OH unit of the dihydroxy substituent with MeO led to significant or total loss of activity. F, Me, NO_2, naphthyl and anthracenyl units also gave inactive compounds.

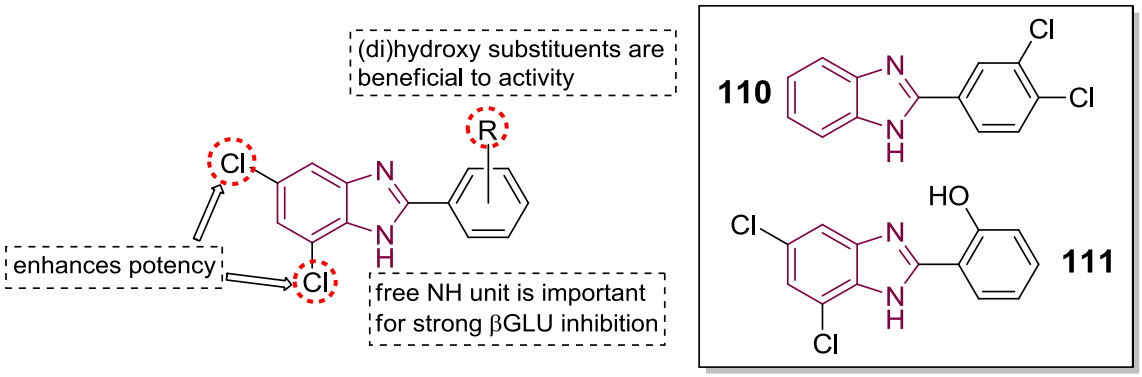


Fig. 22. Benzimidazole based inhibitors and most active compounds.

Molecular docking studies further established that Cl and OH substituents on benzimidazole and phenyl rings respectively are crucial to strong βGLU interaction. The 5,7-dichloro unit on compound **111** afforded hydrophobic interactions with phenyl rings of Tyr504, Tyr508 and side chain methyl of Asn484 and Glu451, while 2-OH substituent interacted *via* strong H-b with NH_2 unit of Lys606 and OH unit of Tyr504. Hydrophobic interactions of phenol ring with Trp587, Asp207 and His385 also aided ligand-receptor stability in the active site.

N-alkylation of benzimidazole with substituted phenacyl units produced βGLU inhibitors with a different trend in potency [194]. Molecules containing EWGs NO_2, Cl and F groups were superior in the series (Fig. 23). Thus, the best activity was found with *ortho*-NO_2 substituted compound **112** (IC_{50} = 4.06 μM) while *meta* and *para*-NO_2 variants exhibited 5 and 2-fold reduced potency respectively. 4-Cl, 4-F and 2,4-diF substituted molecules also had appreciable potencies with IC_{50} = 32.11, 26.30 and 24.75 μM respectively. In contrast, EDGs, 3,4-diOH, 4-phenyl, 4-MeO and 2,4-diMeO gave inferior inhibitors compared to D-SAL (IC_{50} = 48.40 μM).

Akin to imidazole-indole hybrid compound **96** with IC_{50} = 2.10 μM (Fig. 16), molecular hybridization of benzimidazole with amide bond bioisostere, 1,3,4-oxdiazole, resulted in an isopotent hybrid **113** (Fig. 24) with IC_{50} = 2.14 μM [195]. Again, OH-substituted derivatives were stronger inhibitors compared to those containing F, Cl and NO_2, Me and MeO substituents. Docking studies revealed that the molecular hybrids adopted a linear configuration in the active site of βGLU. The oxadiazole nucleus and central-phenyl ring of compound **113** formed strong H-b interaction with NH_2 unit of Asn484 and phenolic OH of Tyr508 respectively. Compound **114** (IC_{50} = 3.14 μM) on the other hand interacted

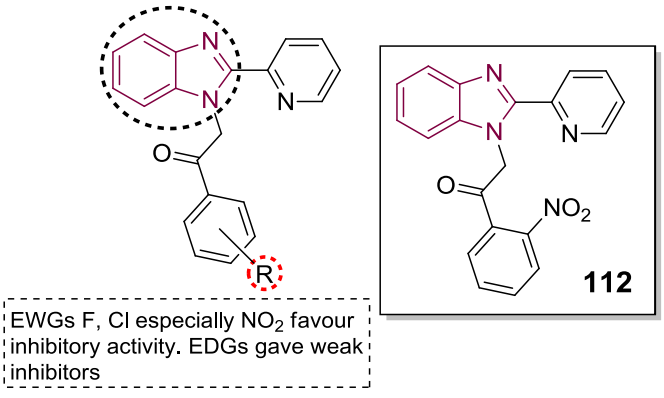


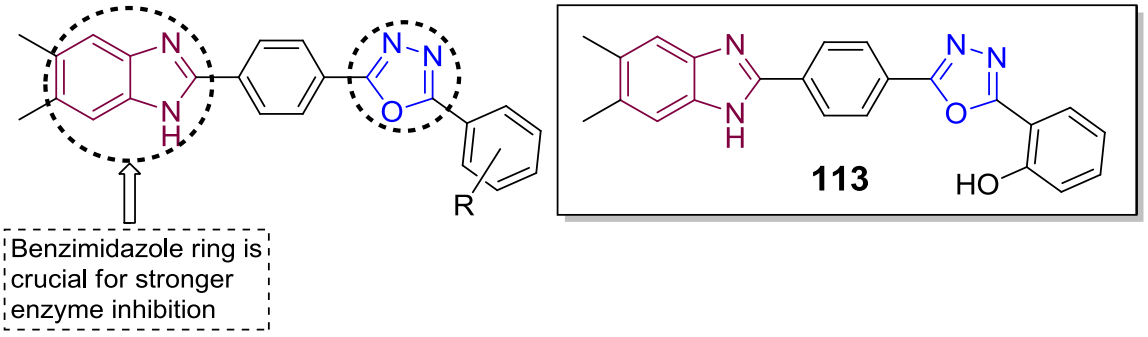
Fig. 23. *N*-Phenacyl-2-pyridylbenzimidazole derived inhibitors and most active compound.

with catalytic acid Glu451 and Tyr508 *via* its central phenyl and phenol rings respectively.

3.2.1.5. 1,3,4-Oxadiazole-based hybrids. In another comparative study using **113** as lead but replacing benzimidazole with benzohydrazone unit (Fig. 25), the importance of benzimidazole nucleus to βGLU inhibitory activity was established by the pronounced loss of activity [196]. Nevertheless, OH-substituted derivatives and benzenetriol compound **115** emerged as the strongest inhibitor with IC_{50} = 7.14 μM. Inhibitory potency was dependent on substituent's position in the order; *para* > *meta* > *ortho* for OH, NO_2 and Cl groups, while the presence of Me or MeO groups gave inactive compounds. Molecular docking studies showed that inhibitory activity correlated strongly with the strength of H-b interaction hence the improved potency seen with OH-substituted derivatives. Notably, the benzenetriol OH unit on compound **115** formed H-b interactions with Glu540, Asp207, Tyr508, His385, and Asn450, while the benzohydrazide unit interacted similarly with Tyr508, Glu540 and Tyr504.

In like manner, replacing the pharmacophores in compound **115** *i.e.* hydroxyl for methoxy and imine for more polar sulfonamide unit (Fig. 26), led to stronger βGLU inhibitory potency for the resulting oxadiazole-benzenesulfonamides [197]. The most potent inhibitors were compounds **116** and **117** with IC_{50} = 2.40 and 6.34 μM respectively; compound **116** showed similar potency as compound **113**. Inhibitory activity was improved for *para*-substituted derivatives, while EWGs produced stronger inhibitors than EDGs. Binding interactions in the active site of βGLU especially H-b interaction of benzamide NH unit with Glu451 established the improved activity of the sulfonamides compared to benzohydrazones. Compound **116** formed H-b interaction *via* sulfonyl oxygens with Asp207 and Asn450, while the benzamide oxygen interacted in similar fashion with Tyr508. Additional ionic bonding between NO_2 groups in compound **117** and Glu451 also accounted for the improved potency.

Molecular hybridization (Fig. 27) of 1,3,4-oxadiazole and its bioisostere 1,3,4-thiadiazole was attempted for βGLU inhibition [198]. However, parallel comparison with other reported oxadiazole-containing hybrids reveal that their hybridization does not produce a remarkable effect on inhibitory potency. Inhibitory potency remained with OH, F and Cl substitutions, while abolished activity persisted when OH unit is replaced with MeO. Evidently, the presence of 2,4,6-trichloro unit significantly contributed to potency. Thus, compound **118** was the most active inhibitor with IC_{50} = 0.96 μM, 2- and 3-fold stronger than 3,4-diOH substituted compound **119** and oxadiazole-sulfonamide hybrid **116** with similar 2,4,5-trichloro unit (IC_{50} = 1.40 μM) respectively. The result partly suggests that significant alteration in molecular lipophilic/



R	2-OH (**113**)	2-F	2-Cl	3-OH	3-F	3-Cl	3-NO_2	4-OH (**114**)	4-NO_2	4-F	4-Cl	2-Pyridyl
IC_{50} μM	**2.14**	19.16	15.14	8.14	16.12	31.14	11.14	3.14	30.14	13.14	32.14	4.12

Fig. 24. Benzimidazole-oxadiazole hybrids and most potent βGLU inhibitors.

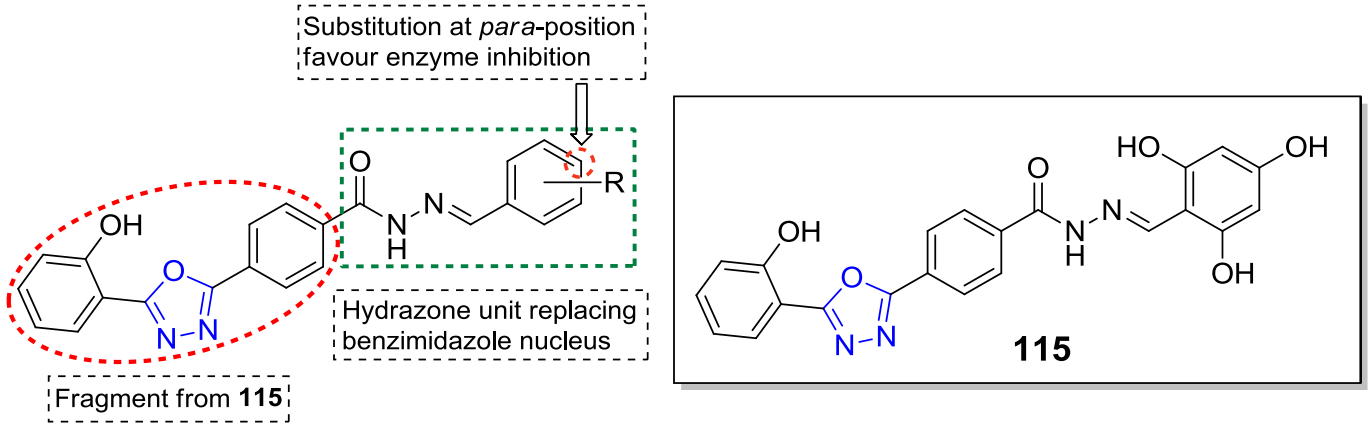


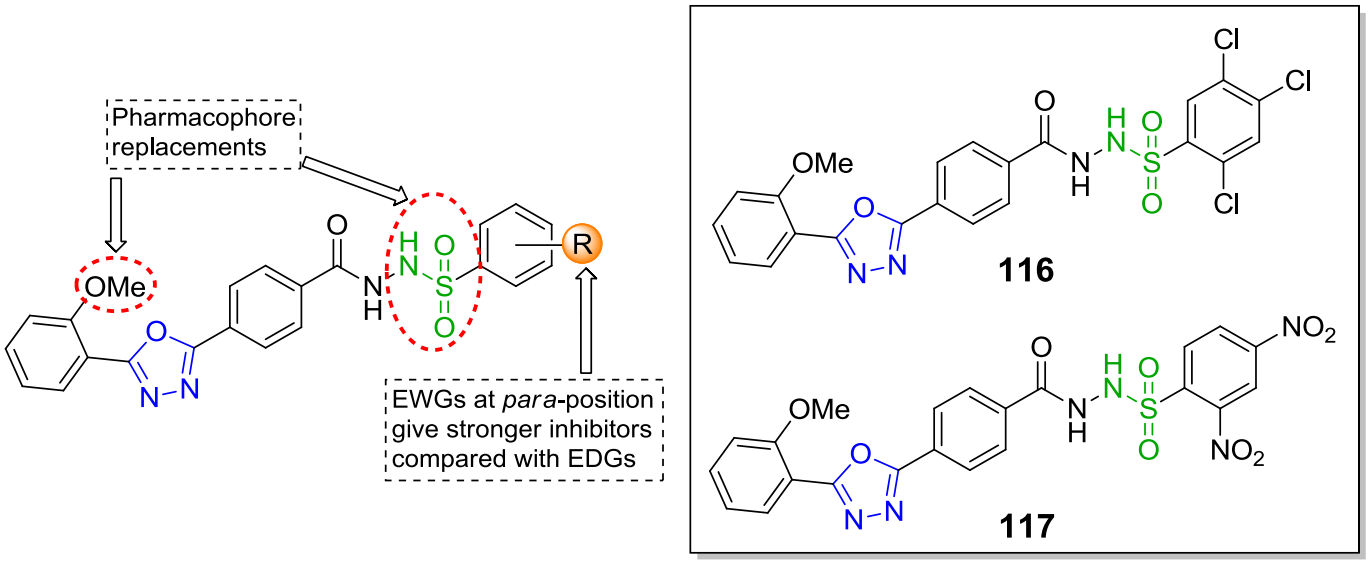
Fig. 25. Oxadiazole-benzohydrazone based βGLU inhibitors and most active inhibitor.

hydrophobic balance and the binding potential of installed pharmacophoric units is crucial to eliciting desirable increase in βGLU inhibitory potency.

3.2.1.6. Benzothiazole-based hybrids. Results of βGLU inhibition studies of 2-arylbenzothiazoles derivatives further established the significance of OH substituent to inhibitory potency, as activity was conserved to OH-substituted compounds [199]. 2,4,5-benzenetriol substituted compound **120** (IC_{50} = 2.26 μM) was the strongest inhibitor in the series with 2- and 21-fold improved potency compared to 2-OH analogue **121** (IC_{50} = 4.23 μM) and standard inhibitor D-SAL respectively (Fig. 28). *In silico* studies of these benzothiazoles revealed that they adopted a linear conformation which allowed appropriate fit into the binding groove of βGLU. *Ortho*-OH unit on compound **120** formed strong H-b interactions with Glu451 while the *para*-OH unit provided H-b interaction with Asp207. The enzyme-inhibitor complex was stabilized in the active site through hydrophobic interactions of benzenetriol ring with indole nucleus of Trp587, His385, Asn484, Tyr504, His509, Arg600, and Lys606. Most importantly, all the active inhibitors were non-cytotoxic in a cytotoxicity assay using mouse embryo fibroblasts (3T3-L1) and Wistar rat hepatocytes (CC-1).

Benzothiazole and benzimidazole are bioisosteres due to their structural and pharmacological similarities; therefore, bioisosteric replacement of one for the other has been consistently explored in drug development. Accordingly, bioisosteric replacement of the benzimidazole moiety in compound **113**, to give new benzothiazole-oxadiazole hybrids resulted in equally potent βGLU inhibitors [200]. The most potent compound **122** (Fig. 29) with IC_{50} = 2.16 μM, was equipotent with compound **113**. Fascinatingly, compound **123** (IC_{50} = 4.38 μM) was also isopotent with similarly substituted compounds **121** and **111**. Further, docking studies of compounds **122** and **123** showed that H-b interactions of phenolic OH units with active site residues *viz*., Glu451, Tyr508, and Asp207, favoured their strong inhibitory potency. Moreover, compound **122** formed hydrophobic interactions with Tyr504 and Lys606 *via* its thiazole core and benzene ring respectively. The reduced potency of compound **123** was established in the strength of these interactions as well as the absence of additional van der Waal contacts of benzothiazole ring in compound **122** with catalytic residues Glu451 and Glu540. *In silico* pharmacokinetic properties modelling of these inhibitors predicted good cell permeability and solubility. The compounds were also non-cytotoxic to 3T3-L1 and CC-1 cell lines.

Following a similar molecular development strategy leading to compound **122**, new benzothiazole-hydrazone hybrids were assembled by deleting the 2-oxadiazolylphenol fragment in compounds **122** and **115** (Fig. 30) [201]. Compared to the parent compound series, the new hybrids had marked reduction in potency. The recurring pattern of reduced or abolished activity also persisted with Me and MeO substituents. Nevertheless, compound **124** was



R	2-Cl	3-Cl	4-Cl	2,4-diCl	2,5-diCl	**2,4,5-triCl**	**2,4-diNO$_2$**	4-F	4-Br	4-NO$_2$	4-MeO
IC$_{50}$ μM	18.38	29.25	12.26	14.55	19.06	**2.40**	**6.34**	11.30	25.06	38.38	76.40

Fig. 26. Oxadiazole-benzenesulfonamides and most active inhibitors **116** and **117**.

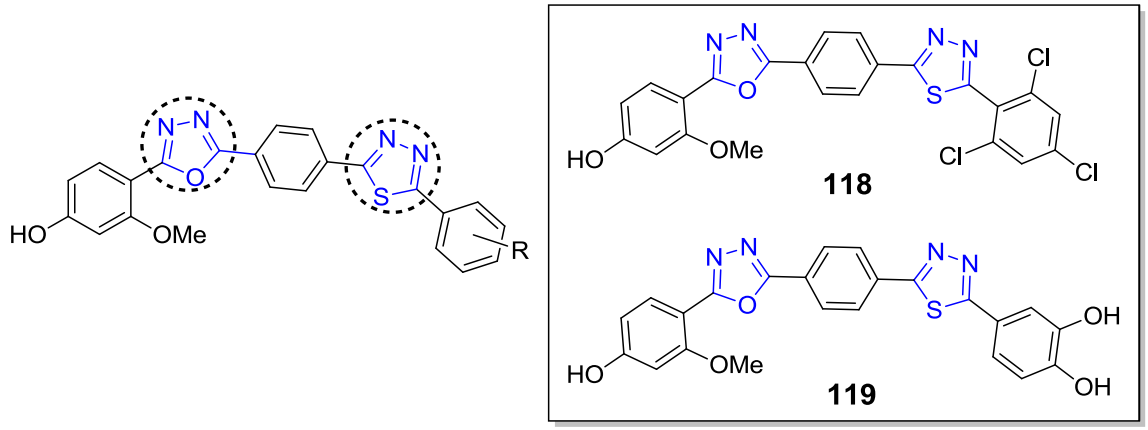


Fig. 27. Most potent oxadiazole-thiadiazole hybrids.

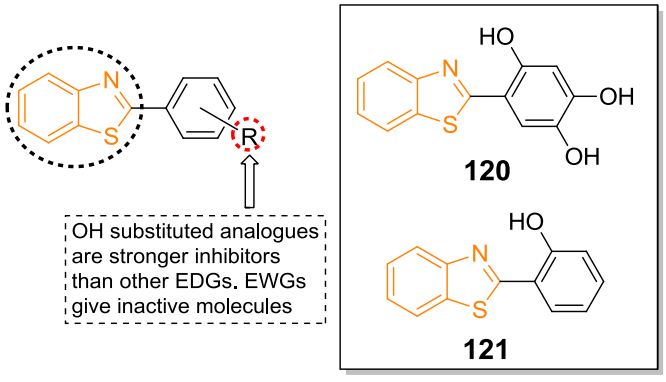
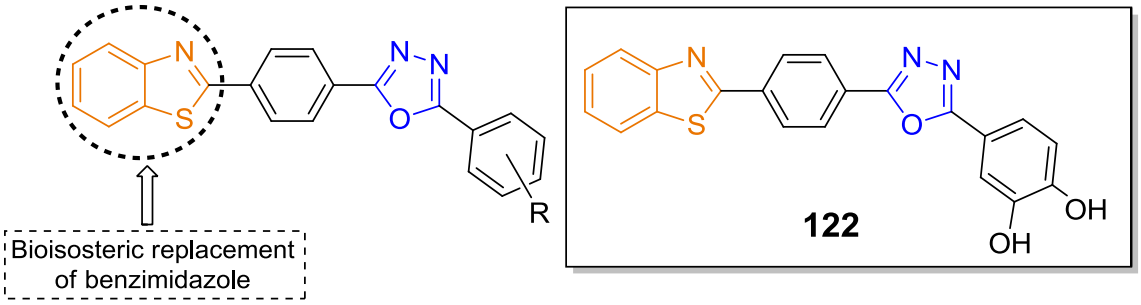


Fig. 28. Most active benzothiazole derived βGLU inhibitors.

the strongest inhibitor in the library with IC$_{50}$ = 16.50 μM. Its reduced activity was further established by the similar but weaker binding interactions compared to compounds **122** and **115**.

3.2.1.7. Triazole. The triazole ring also affords potent βGLU inhibitors. *In vitro* evaluations of 1,2,4-triazole-Schiff bases [202] presented isatin-containing compound **125** (Fig. 31) as the strongest inhibitor with IC$_{50}$ = 2.50 μM and 19-fold superior activity than D-SAL. Cl, OH and NO$_2$ substituents at *ortho*-positions also gave appreciable activities whereas Me, MeO, cumyl and other aryl derivatives were inactive. Docking simulations of compound **125** in the active site of βGLU revealed strong H-b interaction between isatin NH and catalytic residues Glu540 and Tyr504. The phenyl ring also provided π-anion and π-π interactions with Glu451 and Tyr504 respectively while van der Waal contacts with Lys606, Tyr508, Val410 and Asn450 stabilized the inhibitor-βGLU complex. However, amide bond bioisostere, 1,2,3-triazole, is a stronger βGLU



R	2-OH **(123)**	3-OH	2,3-diOH	2,5-diOH	2,4-diOH	3,4-diOH	4-OH	4-F	4-Cl	2-Pyridyl
IC_{50} μM	**4.38**	32.42	14.89	7.20	19.68	2.16	14.42	8.56	15.40	31.2

Fig. 29. Benzothiazole-oxadiazole hybrids and most potent βGLU inhibitors **122** and **123**.

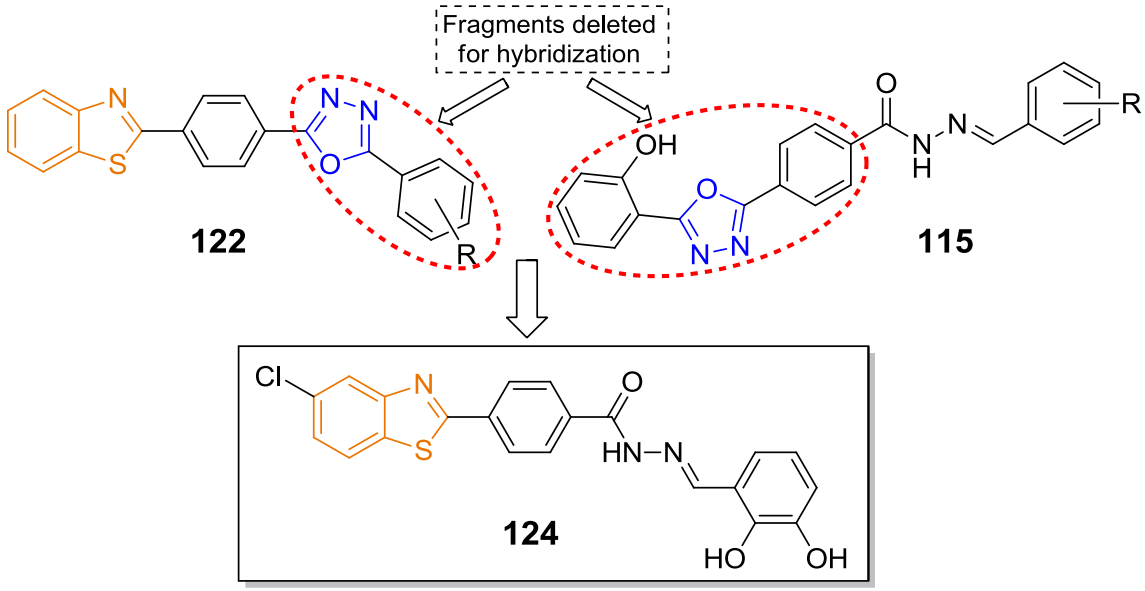


Fig. 30. Molecular design of benzothiazole-benzohydrazone hybrids and most active inhibitor **124**.

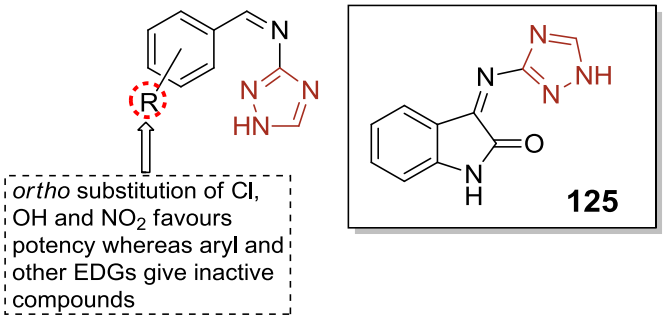


Fig. 31. Most potent 1,2,4-Triazole Schiff base **125**.

inhibitor compared to its 1,2,4-triazole isomer. Molecular hybridization with carbazole [203] delivered eight compounds having $IC_{50} < 3$ μM (Fig. 32). Compound **126** ($IC_{50} = 0.55$ μM) was the most potent hybrid in the series and 83-fold stronger than D-SAL. All the carbazole-1,2,3-triazole tethers were non-cytotoxic to 3T3 cells.

3.2.1.8. Indole-based hybrids. A library of indole analogues containing the hydrazone unit in compound **115** have been examined for βGLU inhibition [204]. Evinced by the increased potency, the indole pharmacophore delivers stronger βGLU inhibitors compared to benzothiazole and oxadiazole. For instance, the most potent inhibitor **127** with $IC_{50} = 0.50$ μM (Fig. 33), was 100-fold more potent than D-SAL and 42-fold stronger than similarly substituted oxadiazole variant from compound **115** library. Compounds **128** ($IC_{50} = 2.40$ μM) and **129** ($IC_{50} = 2.50$ μM) were also 3-fold and 43-fold stronger respectively than their corresponding oxadiazole variants. Moreover, substitution at *ortho* and *para*-positions gave stronger inhibitors compared to *meta*-position, while potency in monosubstituted analogues followed the order; F > OH > Cl > pyridyl > NO_2 > MeO. The excellent potency of compound **127** was afforded by strong H-b interaction of position-5-OH with Glu451 and Glu540 as well as position-4-OH with Tyr508. Indole and benzohydrazone NH units formed H-b interactions with Asn502 whereas the ligand-receptor complex was stabilized by π-alkyl interaction of indole ring with Trp528. Expectedly, the reduced activity of compound **128** was seen in its weaker interactions in the active site of βGLU.

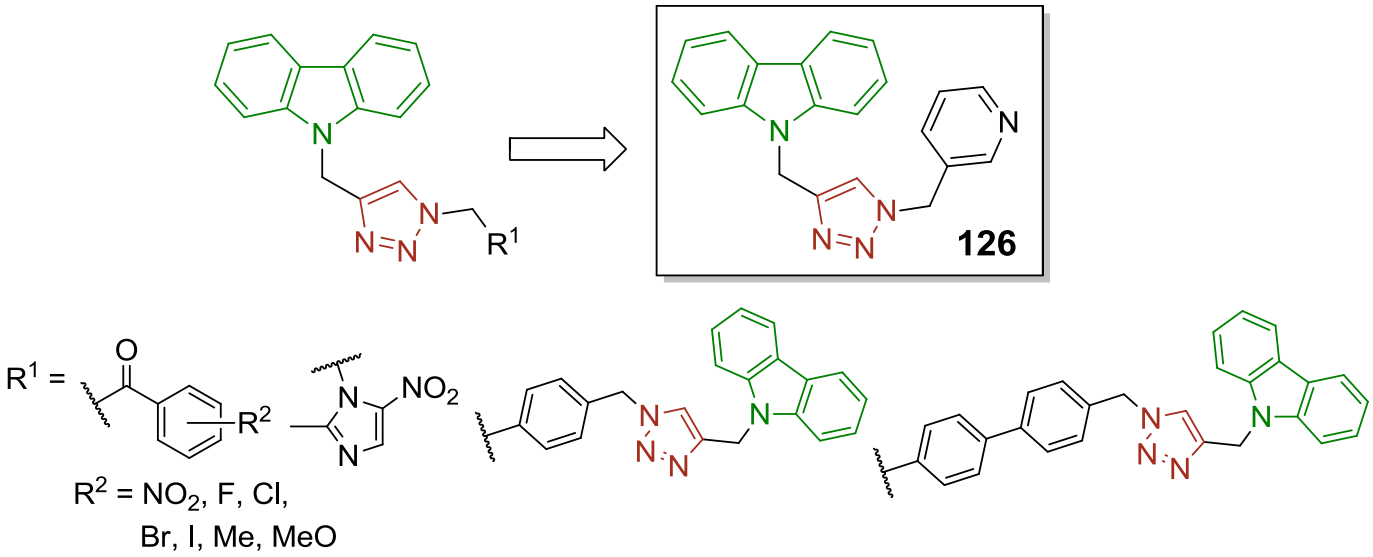
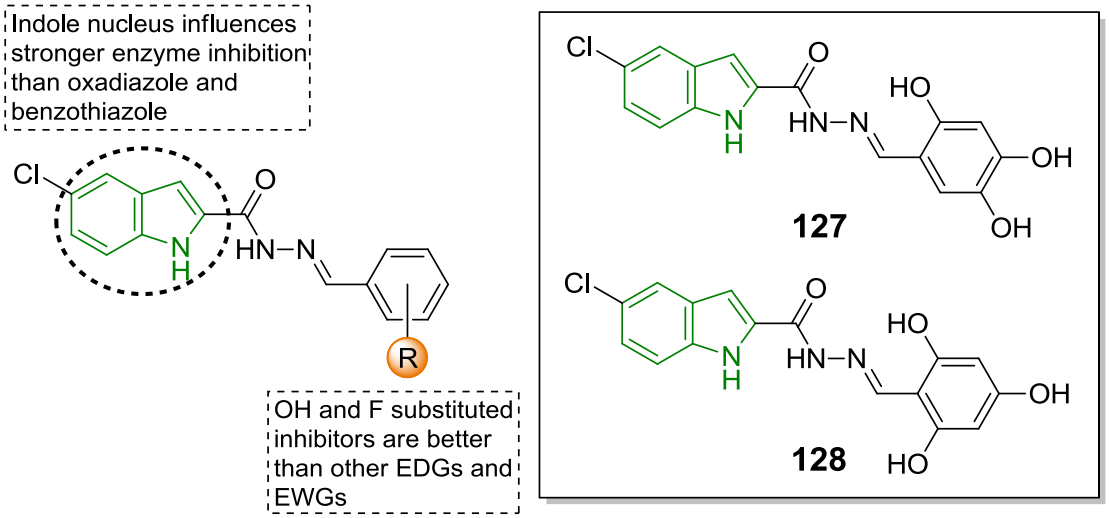


Fig. 32. 1,2,3-Triazole – carbazole tethers and most potent inhibitor **126**.



R	**2-F (129)**	2-Cl	2-NO_2	3-OH	3-F	3-Cl	3-NO_2	4-OH	4-F	4-Cl	4-NO_2	2,4-diOH	2,4-diCl
IC_{50} μM	**2.50**	13.60	32.50	14.40	16.12	53.40	56.50	12.50	6.50	37.20	41.40	8.70	24.60

Fig. 33. Indole-hydrazone hybrids and most active inhibitors **127**–**129**.

Based on the pharmacological significance of thiadiazole pharmacophore and the promising inhibitory activity of indole hybrid compound **127**, hybrids of indole-thiadiazole were assembled as new class of βGLU inhibitors [205]. Generally, thiadiazole nucleus infused stronger inhibitory potencies on the resulting hybrids compared to hydrazone unit. Dihydroxy substituted compound **130** emerged as the strongest inhibitor with IC_{50} = 0.50 μM and equipotent as compound **127**. *Ortho*-substitution was more beneficial to potency than *para* and *meta*-substitutions, while inhibitors' strength followed the order; OH > F > Me > pyridyl > Cl > NO_2 (Fig. 34). However, bioisosteric replacement attempt with oxadiazole proved that thiadiazole unit is preferred for stronger inhibition [206]. The strongest potency remained with 2,3-dihrdoxy substituted derivative **131** (IC_{50} = 0.90 μM), although 2-Cl derivative had an outlying potency.

Bazedoxifene (Fig. 35) is a novel indole-based inhibitor of IL-6/GP130 for the management of triple negative breast cancer [207]. It is also a selective estrogen receptor modulator administered as co-drug with conjugated estrogens (Duavee) in estrogen replacement therapy for the prevention of postmenopausal osteoporosis. Albeit, the high ratio of circulating estrogen metabolites and parent estrogen aided by βGLU deglucuronidation activity in the gut is considered a risk factor for postmenopausal estrogen receptor positive (ER+) breast cancer. Accordingly, a study on the therapeutic utility of this combination drug in an ovariectomized mouse model showed significant reduction in faecal βGLU activity without altering faecal microbiota diversity [208]. The study articulated the significance of bacterial βGLU inhibition to improving the outcome

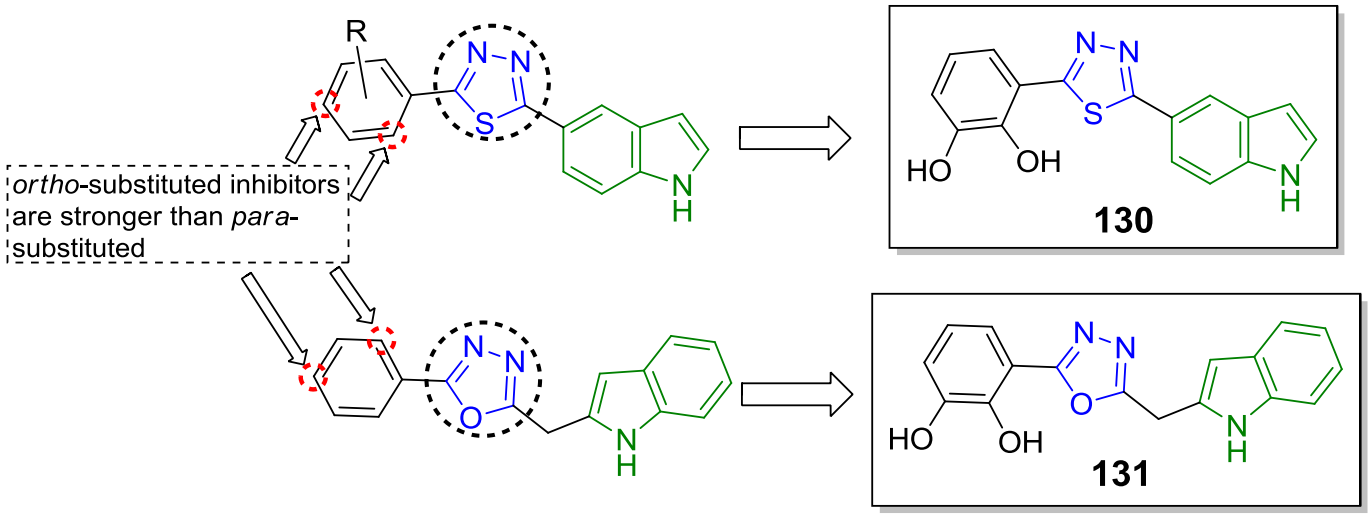


Fig. 34. Indole-thiadiazole/oxadiazole hybrids and most potent compounds **130** and **131**.

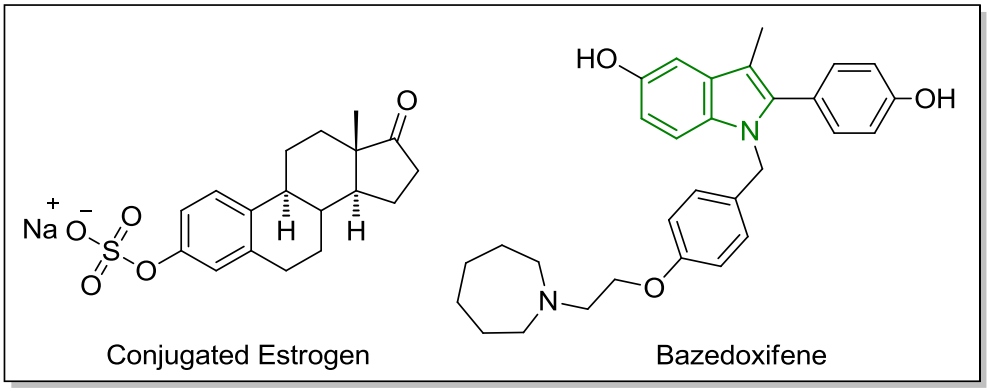


Fig. 35. Combination drug (Duavee) as bacterial βGLU inhibitor.

of long-term administered estrogens for postmenopausal women or breast cancer patients.

Consistent with the excellent inhibitory potencies of indole-based compounds, a library of *bis*-indoles (Fig. 36) have been reported as strong βGLU inhibitors [209]. Once more, the importance of (di)hydroxy substitution to potency was prominent. Compound **132** emerged as the most active molecule with IC_{50} = 1.62 μM and 30-fold superior to D-SAL. *In silico* studies suggested that H-b donor-acceptor potential of OH units on compound **132** significantly influenced ligand-receptor interaction. *Ortho*-OH formed strong H-b with amino acid residues Asp207 and Tyr508, while *para*-OH interacted similarly with His385. Arene-arene interaction of indole ring with Tyr504 also aided the favourable binding to βGLU. In another study [210], both compound **132** and its 2,3-dihydroxy compound **133** (IC_{50} = 1.2 μM) were the strongest inhibitors of bacterial βGLU; albeit with moderate cytotoxicity against 3T3 mouse fibroblasts.

The presence of *N*-phenyl substituted thiosemicarbazide unit however introduced marked increase in βGLU inhibition of 1*H*-bisindoles [211]. Inhibitory potency for monosubstituted analogues followed the order F ≈ CF_3 > Cl > Br > Me > MeO and *ortho*-substitution produced the best result (Fig. 37). Accordingly, 2-F

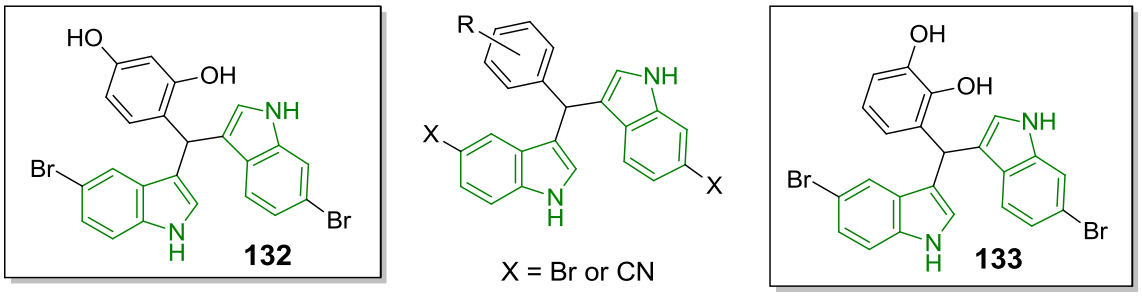
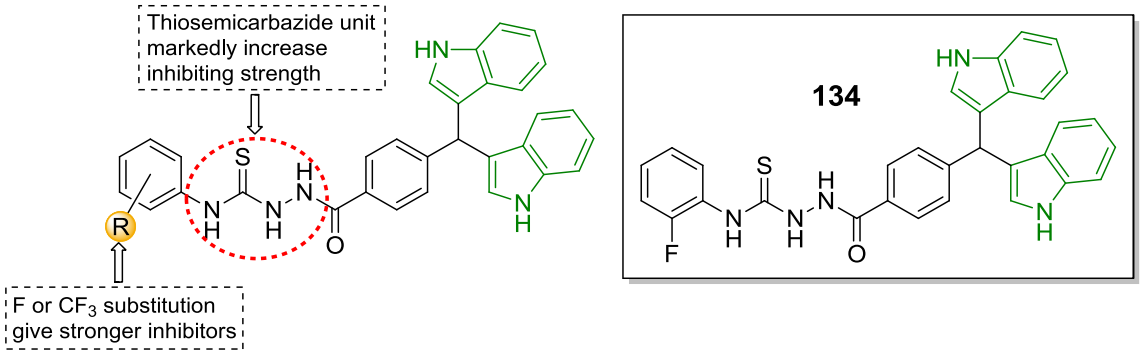


Fig. 36. *Bis*indole based inhibitors and most active molecules **132** and **133**.



R	2-F (**134**)	3-F	4-F	4-CF_3 (**135**)	2-Cl	4-Cl	3,4-diCl (**136**)	2-Br	2-Me	3-NO_2
IC_{50} μM	0.10	2.30	3.10	0.20	0.50	8.50	0.12	2.90	7.12	5.70

Fig. 37. *Bis*indole-thiosemicarbazide conjugates and strongest inhibitors **134**–**136**.

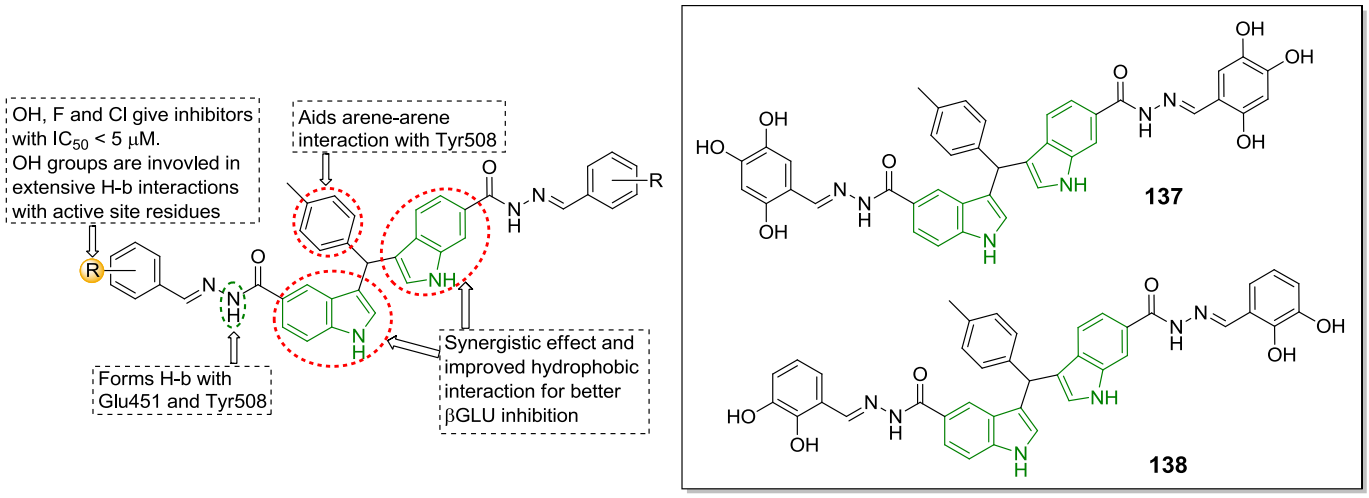
compound **134** emerged as the strongest inhibitor with IC_{50} = 0.1 μM; 484-fold superior potency compared to D-SAL, 2-fold stronger than 4-CF_3 compound **135** and equipotent with 3,4-diCl compound **136**.

Results from *in vitro* evaluation of a library of bisindole-hydrazones against βGLU activity [212], suggested a synergistic effect of two indole nuclei on enzyme inhibition (Fig. 38). Majority of the examined molecules showed IC_{50} ˂ 10 μM; the best result overall for hydrazone conjugates. Identical to compound **127** series, OH and F substituted molecules were stronger inhibitors. Therefore, compounds **137** and **138** were the strongest inhibitors with equal potency; IC_{50} = 0.10 μM. The presence of MeO unit was again unfavourable to activity as seen in the 2- to 11-fold decrease in activity of MeO substituted variants of di-OH derivatives. Docking studies further revealed that an extensive H-b network *via* OH units and hydrophobic interactions *via* indole nuclei fostered the favourable fit of compounds **137** and **138** in βGLU's catalytic pocket for strong inhibition. Benzenetriol OH units of compound **137** acted as H-b donor to Asn502, Gln524, His385, Tyr508 and H-b acceptor to Glu540, Asn450, Lys606, Trp528. Tyr508 also formed H-b interaction with amide NH and arene-arene interaction with phenyl ring of tolyl unit.

3.2.2. Chalcones

Chalcones are both a class of naturally occurring flavonoid family and synthetically obtainable compounds with extensive therapeutic applications [213]. The presence of a highly reactive α,β-unsaturated ketone unit and delocalized electrons makes them coveted precursors for many synthetic manipulations and pharmacological explorations.

The pro-inflammatory role of βGLU in inflammatory disorders motivated the design of a series of (di)hydroxychalcones (Fig. 39), to inhibit the release of βGLU from stimulated rat mast cells and neutrophils [214–216]. It was established that hydroxychalcones were stronger and selective inhibitors of neutrophil-derived βGLU over mast cell-derived βGLU. The best inhibitors were compounds **139** (IC_{50} = 0.6 μM; ˃ 160-fold selectivity) and **140** (IC_{50} = 1.3 μM; 7-fold selectivity). SAR analysis showcased the importance of α,β-



R	2,4,6-triOH	2,4-diOH	2-OH-4-MeO	3,4-diOH	3-OH-4-MeO	2,5-diOH	2-OH-5-MeO	2-OH	2-F	2-Cl	2,4-diCl
IC_{50} μM	**0.20**	**0.30**	3.45	**0.30**	2.67	1.14	2.10	1.50	1.20	2.80	1.10

Fig. 38. Most potent inhibitors, SAR analysis and binding interaction of *bis*-indole-hydrazone tethers.

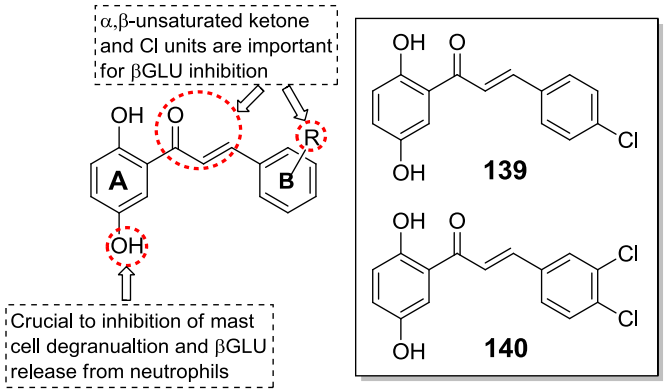


Fig. 39. Dihydroxychalcones and strongest βGLU inhibitors.

unsaturation to βGLU inhibition as dihydro derivatives were 20 to 30-fold less potent than parent chalcones. The OH unit at position-5 of ring **A** was crucial to the inhibition of mast cell degranulation and release of βGLU from neutrophils. *O*-alkylation of hydroxyl units on phenyl rings particularly ring **A** also reduced potency while abolished activity occurred with increased alkyl size.

Compound **141** (Fig. 40) has emerged as the most potent inhibitor with 75% inhibition at 10 μM and only 3-fold stronger than standard salicylic acid, amongst a series of chalcone-Mannich adducts [217]. Adopting a similar molecular design [218], a library of chalcone-benzamides were found with slightly improved potency and SAR analysis established the preference of EWGs over EDGs for better enzyme inhibition. Compound **142** containing similar 3-Br substituent as compound **141**, emerged as the most potent molecule (84.68% inhibition at 1 mM); 3-fold stronger potency than salicylic acid (24.77% at 1 mM). It was also non-cytotoxic in CCK-8 assay in contrast to 2-fold increased cytotoxicity of equally potent 3-CF_3 analogue. Notably, activity modulation by substituting trimethoxy unit for indole was unsuccessful [219], leading to prominent reduction in percentage inhibitory activity of the chalcones (˃ 8-fold).

3.2.3. Coumarins and azacoumarins

Structural development of a coumarin (chromen-2-one) compound **143** (Fig. 41) recovered from virtual screening delivered a set of moderately potent inhibitors of βGLU activity [220]. Superior activity compared to D-SAL (IC_{50} = 48.40 μM) was recorded compounds **144**–**147** with IC_{50} values of 9.90, 11.70, 21.40 and 34.20 μM respectively. In another attempt [221], despite different molecular tunings including polar OH and Cl groups, the coumarin pharmacophore remained inactive against bacterial βGLU. Conversely, structurally isomeric flavenone (chromen-4-one) bearing a pyranose appendage, showed stronger inhibition of bacterial βGLU [210]. Compound **148** (Fig. 42), the most active inhibitor thereof with IC_{50} = 4.50 μM and 10-fold superior potency to D-SAL, was totally non-cytotoxic against 3T3 mouse fibroblasts. *In silico* studies further disclosed the importance of pyranose ring in H-b interactions with active site residues Glu503, Asp161 and Glu413.

Fascinatingly, introducing 1,3,4-oxadiazole pharmacophore to a chromen-4-one scaffold (Fig. 43), led to significant increase in potency [222]. The new hybrids exhibited consistent trend in their potency based on substituent type and position in the order; F > Cl > Me > NO_2 > Pyridyl > MeO and *ortho* > *para* > *meta* respectively. Thus, fluoro substituted derivatives **149** (IC_{50} = 0.80 μM) and **150** (IC_{50} = 1.10 μM) were the strongest inhibitors overall with 60-fold and 44-fold stronger potencies respectively than D-SAL. The binding modes of **149** reiterated the importance of H-b atoms and 1,3,4-oxadiazole's multiple-binding potential to molecular activity. *Ortho*-fluorine atom was a H-b acceptor to Tyr508 and Lys606 whereas Asn484 formed carbon to H-b interaction with chromenone endocyclic oxygen. Hydrophobic interactions of oxadiazole core with Glu451 and Tyr508 as well as 2-fluorophenyl ring with Asp207, Glu540, Trp597, Lys606 stabilized βGLU-inhibitor complex in the active site.

On the other hand, azacoumarins (1*H*-quinolin-2-ones) having a basic nitrogen atom in place of endocyclic coumarin oxygen, are conferred with stronger and selective inhibitory potencies against bacterial βGLU. Plausibly, their admirable activity is provided by the ability to mimic or interfere the oxocarbenium ion-like transition state akin to iminosugars; thus, allowing the alleviation of drug-induced toxicities and prevention of colon carcinomas.

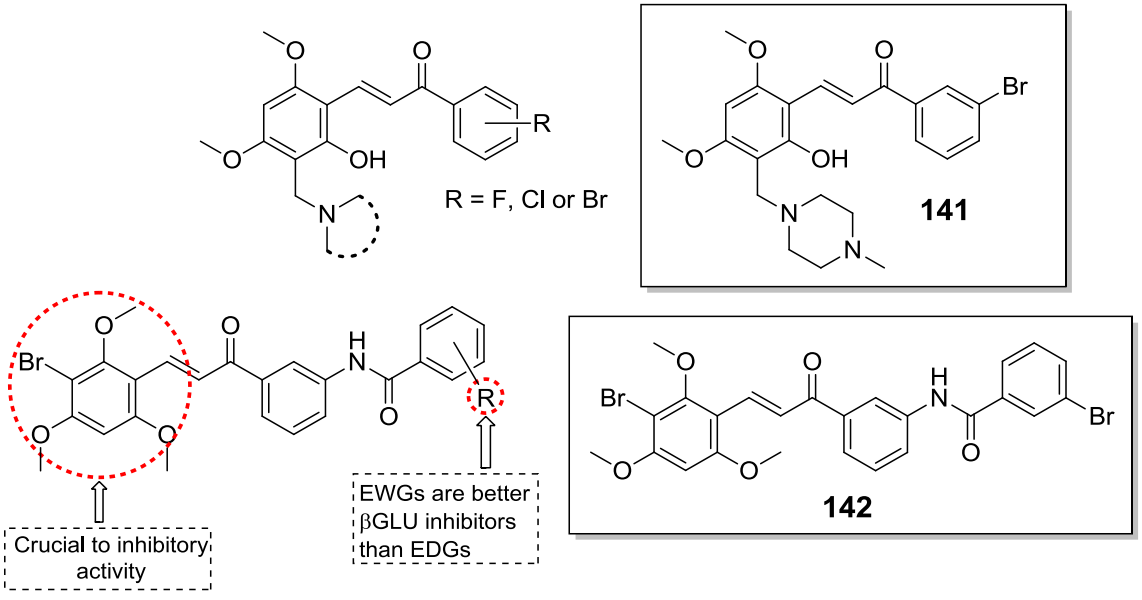


Fig. 40. Chalcone-benzamide tethers and most active inhibitors **141** and **142**.

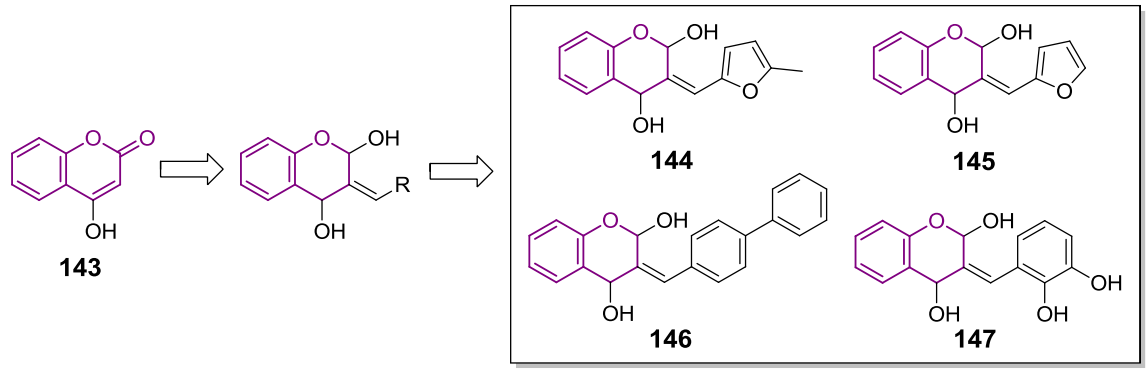


Fig. 41. Most potent chromen-2-one analogues **144** to **147** for βGLU inhibition.

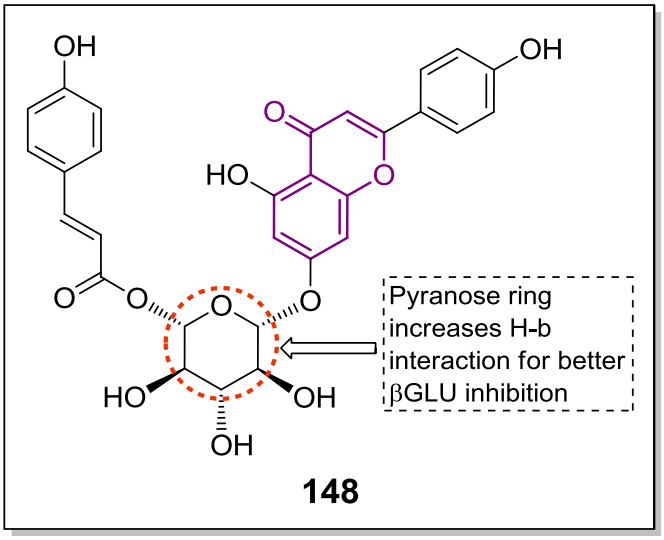


Fig. 42. Most potent chromen-4-one as inhibitor of *E. coli* βGLU activity.

Renowned examples in this class of βGLU inhibitors are the strongly potent azacoumarins **151** and **152** (Fig. 44), identified from high-throughput screening [19,223]. The compounds exhibited *in vitro* IC_{50} values of 0.28 and 0.37 μM respectively, over 1000-fold selectivity for *E. coli* βGLU and 100-fold increased potency than reference inhibitor *glucaro*-δ-lactam **61**. The inhibitors also maintained their potency in living bacterial cells with $EC_{50} = 0.018$ and 0.028 μM. Other similarly active compounds ($IC_{50} \leq 5$ μM) from the screening are presented Table SI. The high selectivity of compounds **151** and **152** for *E. coli* βGLU was established *via* crystal structure resolution of enzyme-inhibitor complex of compound **152**, which showed binding interactions at the entrance of the active site cavity with catalytic residue Glu413, as well as Leu361 and Phe365 in the bacterial loop. No inhibition was observed with an engineered mutant form of *E. coli* βGLU lacking residues 360 to 376.

More importantly, *in vivo* studies of these azacoumarins expresses their remarkable therapeutic potentials. Co-administration of compound **151** at 10 μg (twice daily) with 50 mg/kg of CPT-11 (once daily), alleviated Irinotecan (CPT-11) induced diarrhoea evident by a protected GI epithelium of BALB/cJ mice compared to bloody diarrhoea due to damaged tissues and glandular structure in those receiving CPT-11 alone [19]. Pre-treating C57BL/6J mice with 10 μg of inhibitor **151** before intraperitoneal administration of ulcerogenic dose of NSAIDs – diclofenac (60 mg/kg), indomethacin (10 mg/kg) and ketoprofen (100 mg/kg), also successfully protected the mice models against NSAID-induced small intestine mucosal damage [224,225]. Moreover, compound **151** reduced all enteropathy parameters by inhibiting GI bacterial βGLU-mediated hydrolysis of NSAID-acyl glucuronide in a concentration-dependent manner ($IC_{50} \approx 0.164$ μM). Interestingly, the compound did not alter drug's systemic exposure, hepatobiliary excretion of glucuronides and GI microbiota diversity. Although it possesses a short half-life (˂1 h) and pan-cytochrome P450-mediated poor bioavailability (21%). However, an attempt to reduce the inhibitor's serum exposure for increased GI residence by replacing ethoxy unit with hydroxyl or morpholinyl groups led to inferior pharmacological profile compared to parent compound **151** [226]. Further, compound **152** exerted 2-fold reduction in the severity of diclofenac-induced anastomotic leakage, without affecting drug's plasma concentration in Wistar rats receiving 0.8 μg of the inhibitor and 3 mg/kg of diclofenac [227]. In contrast, rats receiving diclofenac only suffered severe leakage thus suggesting the inhibitor's potential clinical utility to improve anastomotic healing.

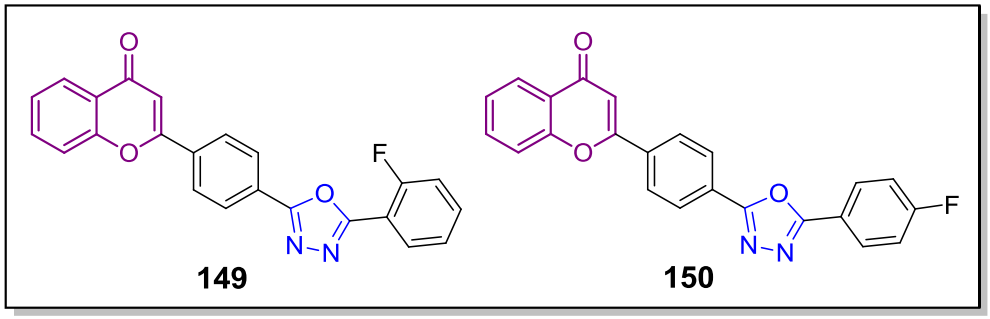


Fig. 43. Most potent chromen-4-one – oxadiazole hybrids for βGLU inhibition.

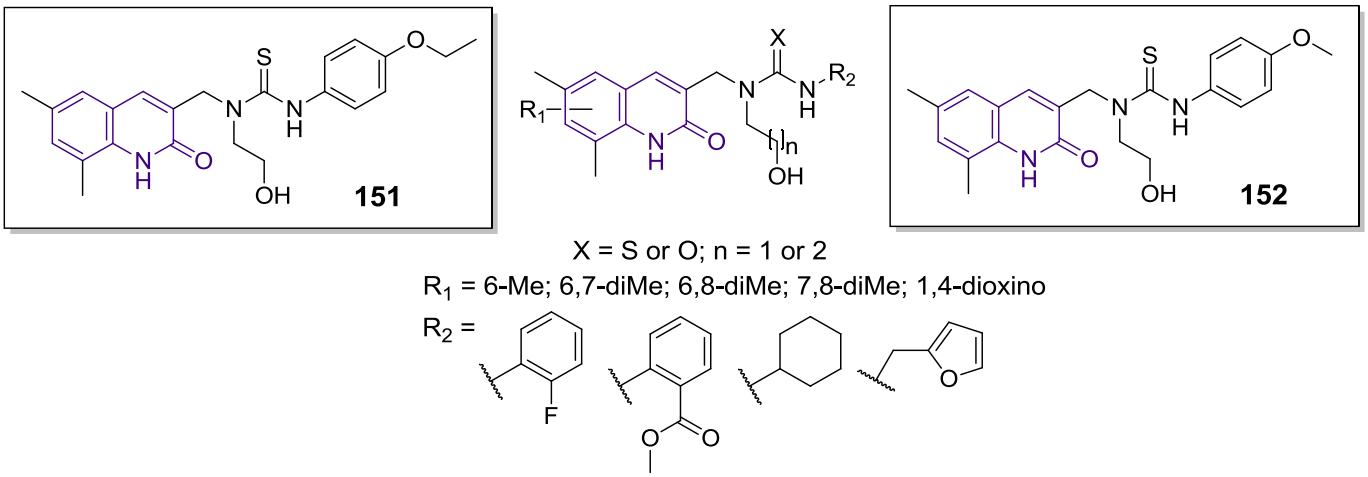


Fig. 44. Azacoumarins and strongest *E. coli* βGLU inhibitor **151** and **152**.

3.2.4. Piperazines

Emerging from the screening protocol which birthed compound **151**, three piperazine-containing compounds **153**, **154** and **155** (Fig. 45) showed promising inhibitory potentials against bacterial βGLU [228]. Though with slightly weaker inhibitory strengths (IC_{50} = 0.54, 8.89, 6.43 μM respectively), their pharmacological profile *viz*., potency in living bacterial cells, selectivity for *E. coli* βGLU over mammalian βGLU or other related glycosidases and non-cytotoxicity to bacterial or human epithelial cells, were identical to compound **151**. X-ray crystallography of compound **155**-*E. coli* βGLU complex revealed similar binding interactions with active sites as compound **151.** The most active compound **153**, equally alleviated CPT-11 induced GI toxicity (bloody diarrhoea) in BALB/cJ mice when co-administered (10 μg/day) by oral gavage with CPT-11, without affecting the anticancer drug's systemic pharmacodynamic properties measured by weight loss.

Detailed kinetic analysis, enzyme inhibition and X-ray crystallography studies of piperazines **156** (IC_{50} = 0.12 μM) and its synthetic analogue **157** (IC_{50} = 0.080 μM) [229], disclosed their mechanistic behaviour as substrate-selective, slow-binding inhibitors of gut bacterial βGLU with similar pharmacological profile as compound **151**. Compounds **156** and **157** both exerted their inhibitory activities by targeting βGLU-glucuronic acid intermediate to form inhibitor-glucuronide conjugate even in the presence of CPT-11 glucuronide hence disrupting the enzyme's catalytic cycle. SAR probing of compound **156** revealed the importance of nucleophilic NH on piperazine unit to potency. Both mono and dimethylated analogues were 80 to 120-fold weaker respectively than parent compounds **156** and **157**. The piperidine derivative having methylene unit for NH was also totally inactive. Moreover, evaluating βGLU inhibitory potentials of clinically available drugs containing piperazine or piperidine units *viz*., Palbociclib, Crizotinib, Vortioxetine, Amoxapine and Ciprofloxacin also established a similar substrate-dependent, high *in vitro* and cell-based potency against enterobacterial βGLU. Amoxapine was the most potent inhibitor with IC_{50} = 0.53 μM. The results consequently rearticulates similar studies [230–233] proposing the repurposing of approved drugs (Fig. 46) as potent inhibitors of βGLU in GI microbiota, to improve the therapeutic efficacy of CPT-11.

3.2.5. Pyridinone, pyrimidinones and quinazolinone

The therapeutic significance of fused pyridinone-furans as anti-inflammatory drug candidates *via* inhibition of neutrophil-derived βGLU has been reported [234]. Compounds bearing MeO and CF_3

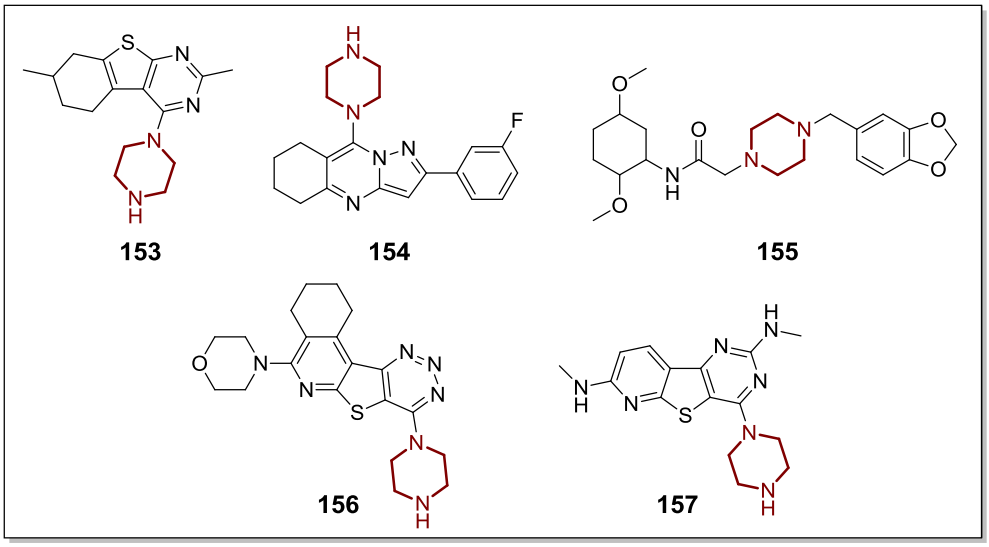


Fig. 45. Piperazine-containing scaffolds as potent *E. coli* βGLU inhibitors.

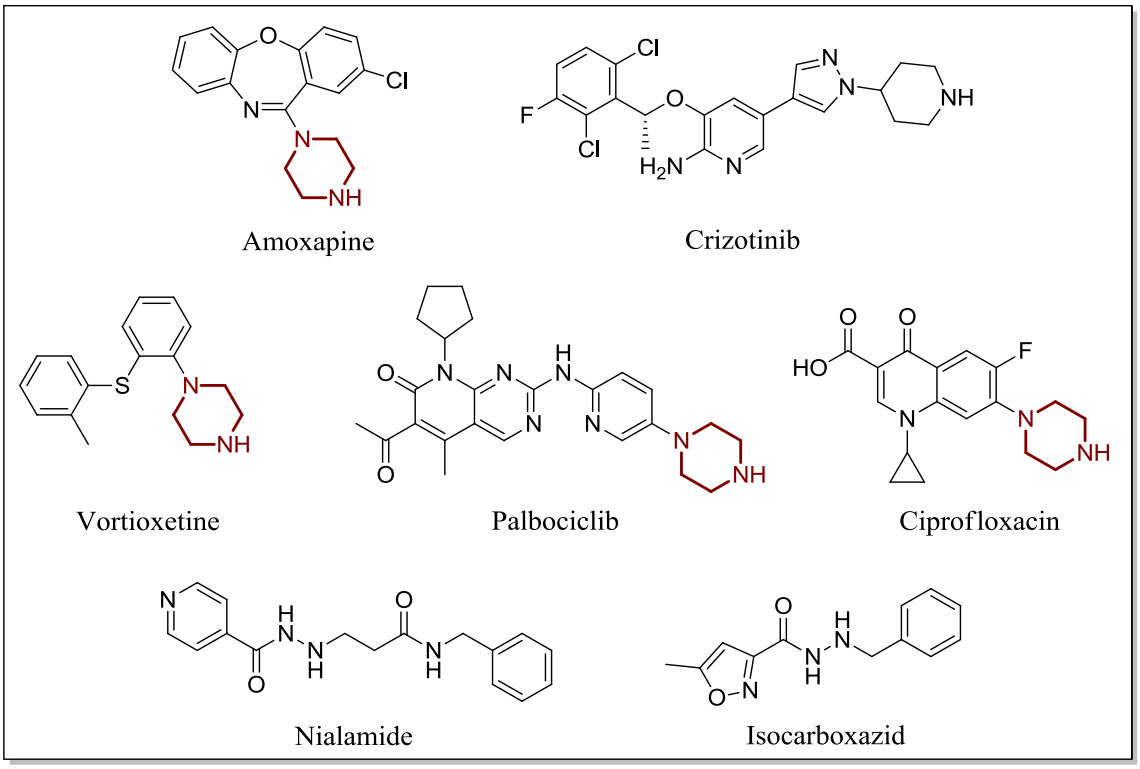


Fig. 46. Repurposable drugs for *E. coli* βGLU inhibition.

substituents on ring **B** (Fig. 47) showed over 70% inhibition at 1 μM with no cytotoxic effects (<32% at 10 μM) in CCK-8 assay as compared to reference salicylic acid (25% inhibition). Replacing ring **B** with furan, thiophene or pyridine however gave poor inhibitors (<45% inhibition). Compound **158** was the most potent with 75% inhibition.

The inhibitory potencies of dihydropyrimidinone carboxylates (Fig. 48), against bovine liver-derived βGLU has reiterated that small polar substituents are necessary for strong enzyme inhibition [235]. Appreciable potency (IC_{50} ˂ 20 μM) was exclusive to CF_3 ˃ F ˃ OH ˃ thienyl ˃ Cl substituted compounds in that order. Whereas, MeO, Br, benzyloxy, furanyl, aliphatic alkyl or aryl units either reduced potency when co-substituted with polar F or OH groups,

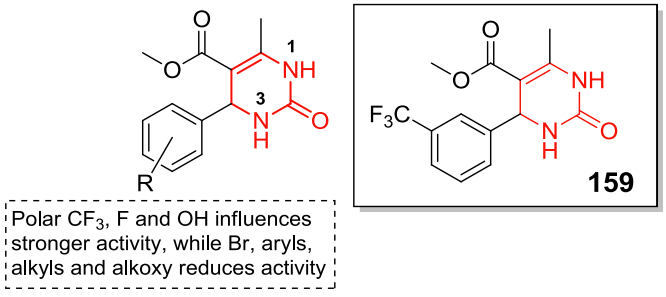


Fig. 48. Pyrimidinone-based inhibitors and most active compound.

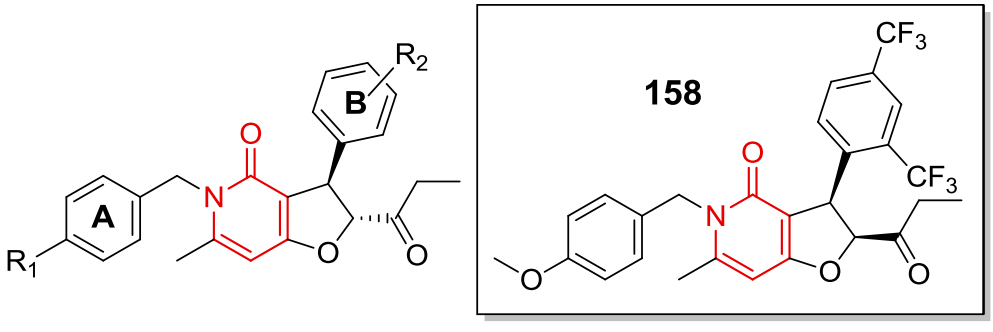


Fig. 47. Fused pyridinone-furans as anti-inflammatory agents *via* βGLU inhibition.

gave inferior activity with monosubstitution or rendered the molecule totally inactive. Therefore, the most active compound **159** with IC_{50} = 9.38 μM, found favourable fit in the active site of βGLU to form strong H-b interactions with key residues. NH of Asp207 was both a H-b donor and acceptor to pyrimidinone carbonyl oxygen and free NH at position-1 respectively. Carboxylate carbonyl oxygen interacted similarly with Arg600 whereas position-3 NH interacted with catalytic residues Glu451 and Tyr508. However, pyrimidinetriones (barbituric acid) [236,237], exhibited inferior potencies compared to standard D-SAL (IC_{50} = 45.75 μM), despite their similar binding interactions as compound **159** with active site residues and non-cytotoxicity to 3T3 cells.

In a predating study, the structural homolog of pyrimidinone, quinazolinone [238], exhibited a unique trend in inhibitory potency against *E.Coli* βGLU with significant tolerance for lipophilic alkoxy groups, although poor activity with methyl substituent persisted. The most active compound **160** (Fig. 49) bearing 3,4-diMeO unit had IC_{50} = 0.60 μM, 76-fold superior potency to D-SAL and equipotent with 2-EtO substituted analogue **161** (IC_{50} = 0.70 μM). Intriguingly, replacing alkoxy units with more polar OH, NO_2 and Cl substituents led to significant reduction in potency. Inhibitory potency therefore followed the order; MeO ˃ EtO ˃ $N(Me)_2$ ˃ NO_2 ˃ OH ˃ Cl and *ortho* ˃ *para* ˃ *meta*. Importantly, the quinazolinone-based inhibitors were all non-cytotoxic to 3T3 cells. It is also noteworthy that a quinazolinone compound (Table SI) from Ref. [223], also showed strong inhibitory potency (IC_{50} = 1.90 μM) against bacterial βGLU.

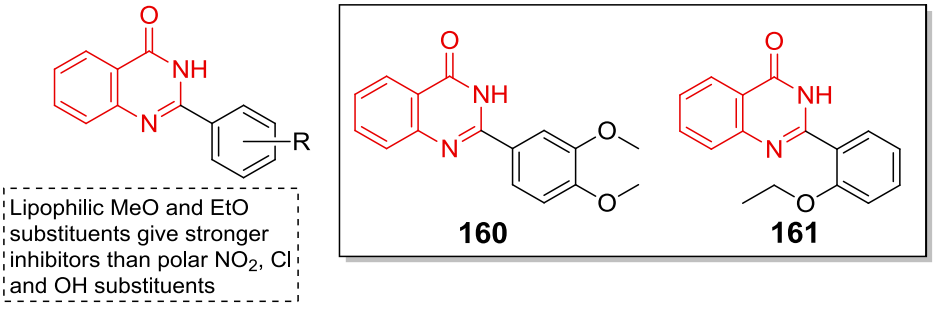
3.2.6. Quinolines

The quinoline nucleus can be regarded as a household name in medicinal chemistry, due to its inexhaustible pharmacological application and occurrence in many clinically important natural products and synthetic molecules. The desirable pharmacokinetic profile and ease of accessibility with wide substrate tolerance for different synthetic protocols, also encourages its utility in countless synthetic explorations. Research endeavours aimed at quinoline-based βGLU inhibitors are therefore presented in this section.

Quinoline-3-carbohydrazides [239], prepared analogously as hydrazone tethers above also holds promising inhibitory activities. Similarly, OH unit at *ortho*-position yielded stronger inhibitors than F > Cl > NO_2 > Pyridyl > thiophenyl > furanyl ≈ Me > MeO, in that order. Moreover, *ortho*-substitution consistently gave superior inhibitors compared to *para*-position, while *meta*-substitution conferred the weakest potency. 2 to 3-fold reduction in potency persisted when OH unit is substituted for MeO unit (Fig. 50). Hence, compound **162** (IC_{50} = 2.11 μM) and **163** (IC_{50} = 2.60 μM) emerged as the most potent inhibitors overall. Docking studies revealed that OH unit at position-4 of quinoline ring fostered important H-b interaction with catalytic acid Glu451, while carbonyl unit of hydrazone moiety formed H-b interaction with NH of Tyr504.

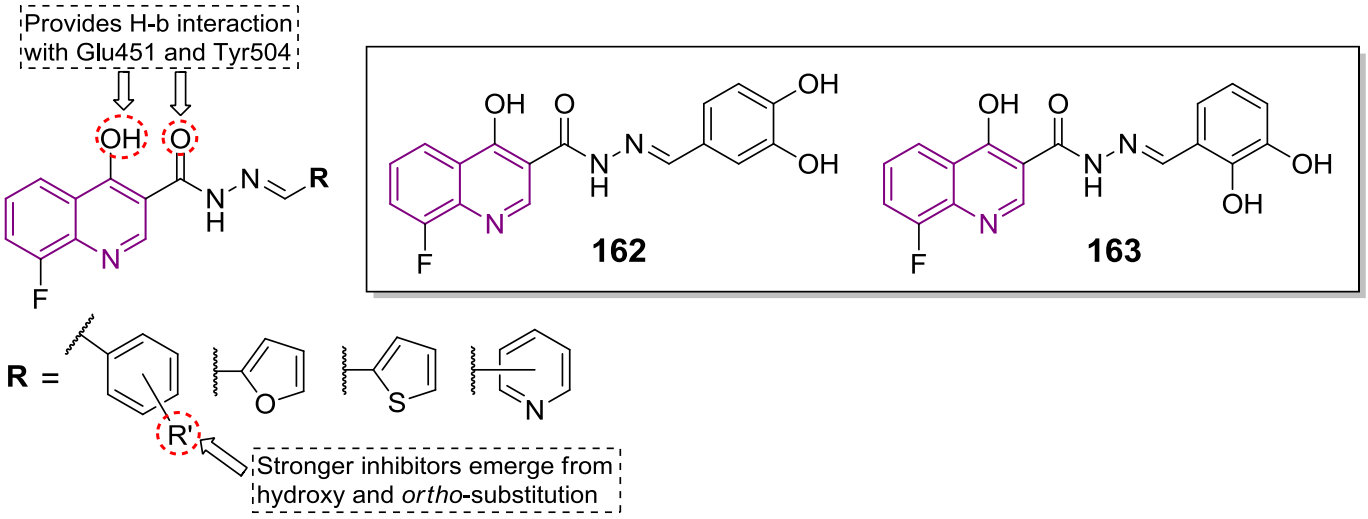
In another quest for new anti-inflammatory agents, quinoline-furan hybrids (Fig. 51) were designed as inhibitors of βGLU release from activated neutrophils [240]. SAR analysis, established that 2-(furan-2-yl)quinolines (**Type A**) were stronger inhibitors of inflammatory mediators than tricyclic furo[2,3-*b*]quinolines (**Type B**). Whereas, formyl unit favoured potency compared to acetyl, oxime, methyl oxime as well as both α,β-unsaturated butan-2-one and its saturated variant. Therefore, non-cytotoxic compound **164** (IC_{50} = 5.0 μM) was the strongest inhibitor of βGLU release with 3-fold superiority to reference trifluoperazine. Acetyl isomer compound **165**, also had similar non-cytotoxic and strong inhibitory potency (IC_{50} = 7.5 μM).

Akin to azacoumarins (1*H*-quinolin-2-ones) **151** and **152**, new quinoline-pyrazole conjugates were prepared *via* hit development protocol of compound **166** (Fig. 52) identified from high-throughput screening, for the inhibition of intestinal bacterial βGLU's hydrolytic activity on SN-38G fo alleviate CPT-11 drug-induced toxicity [241,242]. Interestingly, all the resulting derivatives showed good selectivity for *E. coli* βGLU except 2-Cl, 3-OH, 3-Me and 4-NH_2 substituted derivatives. The trend in inhibitory potency, based on substituent's position varied in the order; *meta* < *ortho* < *para*, while the presence of strongly hydrophilic OH, NO_2 and NH_2 units at *para*-position was detrimental to potency. Consequently, 4-CF_3, 4-Cl, 4-F, 4-COMe and 4-Me substituted derivatives with IC_{50} = 98, 35, 140, 130 and 37 nM respectively, exhibited the highest selectivity and % inhibition of *E. coli* βGLU. Although installing F, Cl, Br or Me substituents on quinoline pharmacophore increased potency for 4-Cl and 4-Me compounds, cytotoxicity at 100 μM to *E. coli* cells thwarted their therapeutic utility as well as other potent inhibitors (4-CF_3, 4-Cl and 4-COMe) in the study. Only 4-F and 4-Me substituted compounds **167** and **168** respectively, remained non-lethal to *E. coli* cells. In addition, compound **167** showed greater inhibition of intestinal βGLU (75%) following oral administration to BALB/cJ mice for 5 consecutive days compared to compound **168** (40%). Whereas, pharmacokinetic profiling of compound **168** revealed its lower plasma concentration and increased GI residence for improved intestinal βGLU inhibition compared to azacoumarin compound **151**. Most importantly, oral co-administration of compounds **167** or **168** at 3 mg kg^{-1} day^{-1} for 10 consecutive days with 50 mg kg^{-1} day^{-1} intraperitoneal



R	3,4-diMeO	3-EtO-4-OH	3,4-diOH	3-OH-4-MeO	2-OH-5-MeO	2,5-diOH	2-EtO	2-NO_2	2-Cl	2-OH	4-MeO	4-EtO	4-NO_2	4-OH
IC_{50} μM	0.60	1.17	2.10	2.80	2.10	3.20	0.70	1.50	1.80	10.0	1.10	2.10	20.10	22.2

Fig. 49. Quinazolinone-based *E. coli* βGLU inhibitors and most active inhibitors **160** and **161**.



R	2,4-diOH	2-OH-4-MeO	2,5-diOH	2-OH-5-MeO	2-OH	3-OH	4-OH	2-F	2-NO_2	4-Cl	2-pyridyl
IC_{50} μM	3.10	9.60	5.01	2.10	6.70	18.10	9.60	9.20	16.60	18.40	19.40

Fig. 50. Quinoline-hydrazone tethers and most active compounds **162** and **163**.

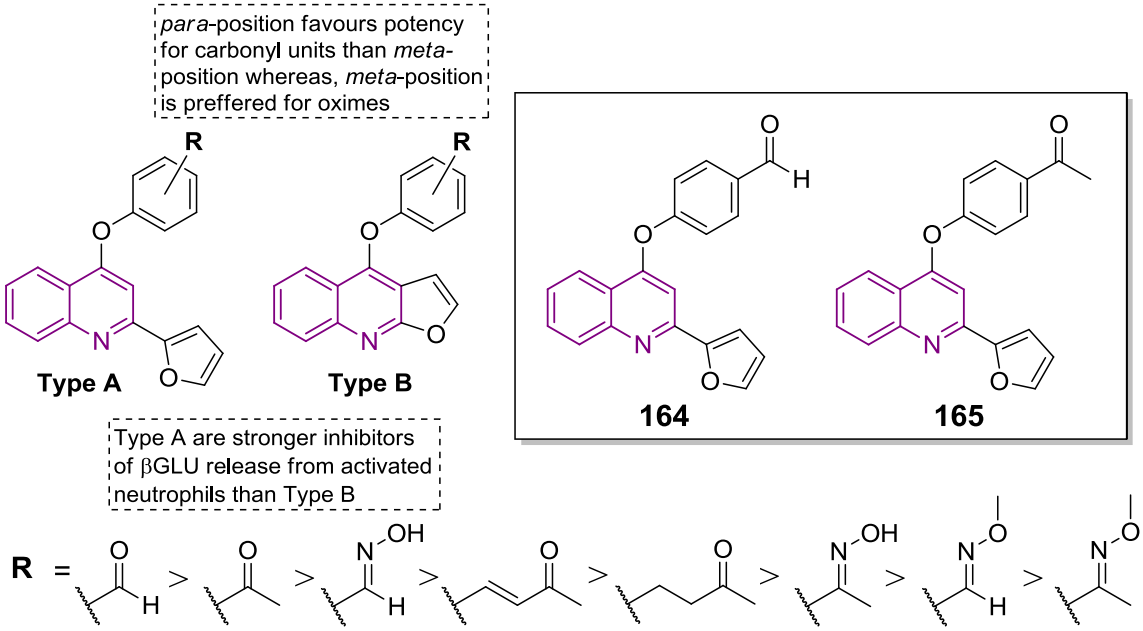


Fig. 51. SAR and most active fused quinoline-furan hybrids **164** and **165**.

injection of CPT-11, protected BALB/cJ mice models against CPT-11 drug-induced intestinal mucosal injury, without altering drug's therapeutic efficacy. Furthermore, mechanistic studies established that the presence of electronegative and electroneutral surface charges on *E. coli* active site pocket and the protonation of *N*-1 and *N*-5 atoms avails a pH-dependent and selective inhibition for the compounds (Fig. 52). Consequently, compounds **167** and **168** are clinically viable agents for protecting against GI *E. coli* βGLU-mediated mucosal damage and preventing the release of chemical carcinogens crucial to the development of precancerous lesions for colon carcinogenesis.

3.2.7. Other synthetic inhibitors

The 200-fold increased inhibitory potency of benzohydrazide–*N*-cyanoethyl tethers [243], compared to parent compounds articulates the significance of a balanced molecular lipophilicity to βGLU inhibition. *O*-alkylation with benzyl, benzoyl or tosyl groups slightly influenced potency for OH-substituted units; benzylation produced the best result (Fig. 53). Hence, **169** (IC_{50} = 1.60 μM) and **170** (IC_{50} = 2.20 μM) were the most active conjugates in the series. Similarly, phenoxyacetohydrazones [244] have displayed 2 to 5-fold improved potency compared to D-SAL (IC_{50} = 48.40 μM). Compounds **171** and **172** (Fig. 54) emerged as the

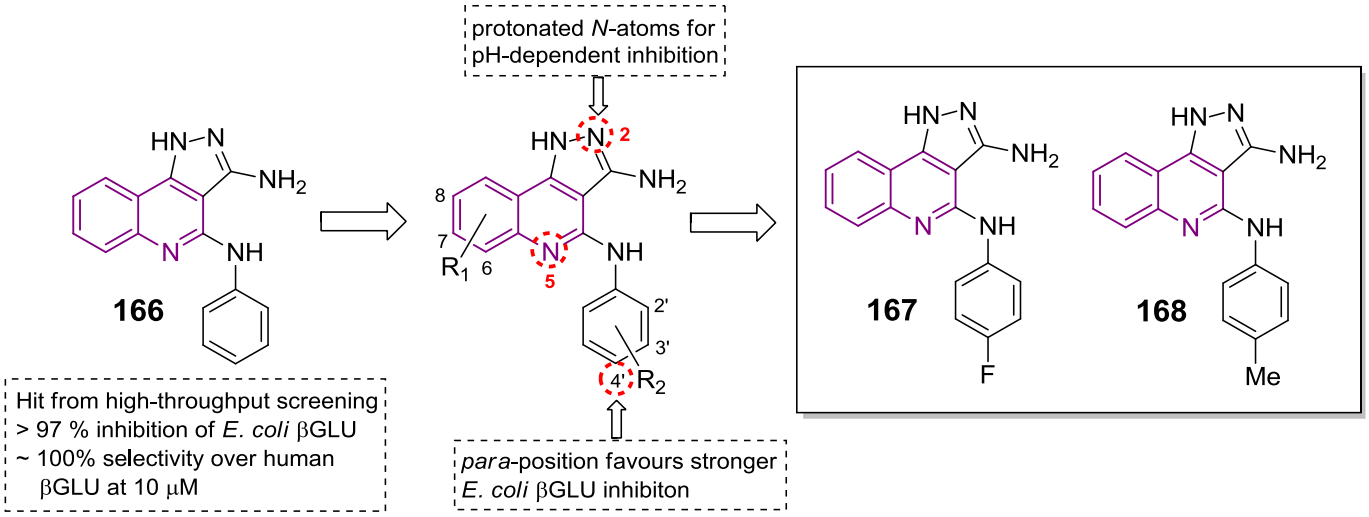


Fig. 52. Hit development, SAR and clinically useful quinoline-pyrazole conjugates **167** and **168**.

strongest inhibitors with IC_{50} values of 9.20 and 9.47 μM respectively. However, parallel comparison with other hydrazone tethers revealed that the phenoxyacetohydrazones are generally weaker βGLU inhibitors.

The desirable therapeutic significance of fused thienothiophenes has stimulated the synthetic exploration of a thienothiophene skeleton **173** (Fig. 55) for bacterial βGLU inhibition [245]. From the resulting thienothiophene library with interesting structural diversity, only compound **174** showed inhibitory activity against bacterial βGLU with IC_{50} value of 0.9 μM; 51-fold superior potency to D-SAL. Notably, the predicted pharmacokinetic properties using Molinspiration and Osiris calculations suggested that the inhibitor possesses good bioavailability and no toxicity risk as bacterial βGLU inhibitor.

In vitro study of phenyl disulphide–benzenesulfonamide tethers [246], disclosed compounds **175** and **176** (Fig. 56) as potent inhibitors of βGLU (IC_{50} = 2.20 and 3.34 μM respectively), analogous to similarly substituted oxadiazole-benzenesulfonamide compounds **116** and **117** (Fig. 28). Interestingly, bulkier phenyl disulfides **175** and **176** shared similar potency with lower molecular mass thiosulfinate **52**, Fig. 9 (IC_{50} = 3.60 μM). However, substitution at *ortho*-position gave stronger inhibitors than *para*- and *meta*-positions, in contrast to compounds **116** and **117** series. Docking studies further revealed that the strong inhibitory potency of compound **175** is aided by the H-b interactions of sulfonamide NH and oxygen with Tyr205 and Asp207 respectively.

The efficacy of urea unit for βGLU inhibition was also examined

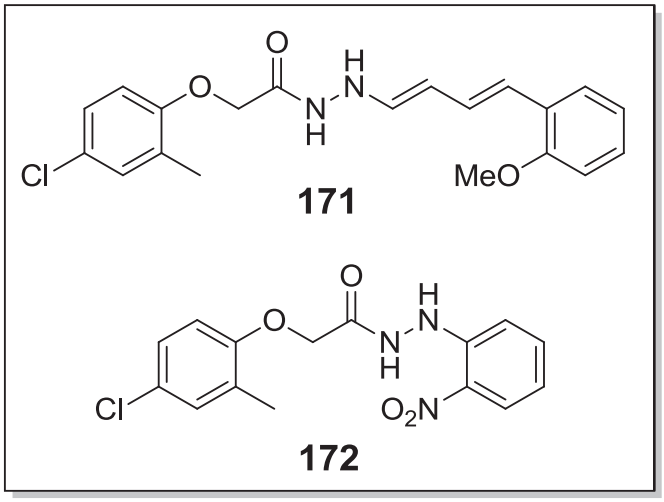


Fig. 54. Strongest phenoxyacetohydrazone-based βGLU inhibitors.

using a library of NO_2 substituted *N*-phenyl ureas [247]. Therefrom, appropriate positioning of polar NO_2 unit was important to inhibitory potency of examined molecules. Compound **177** (Fig. 57), emerged as the only inhibitor with promising activity with IC_{50} value of 3.38 μM. However, thiourea analogues bearing *meta*-Cl substituent on *N*-phenyl ring [248], were stronger inhibitors

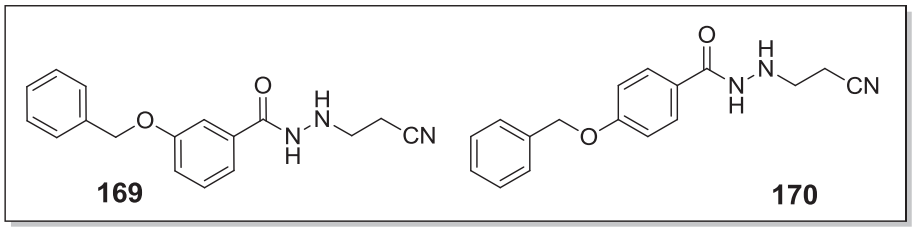


Fig. 53. Most potent benzohydrazide – *N*-cyanoethyl tethers against βGLU.

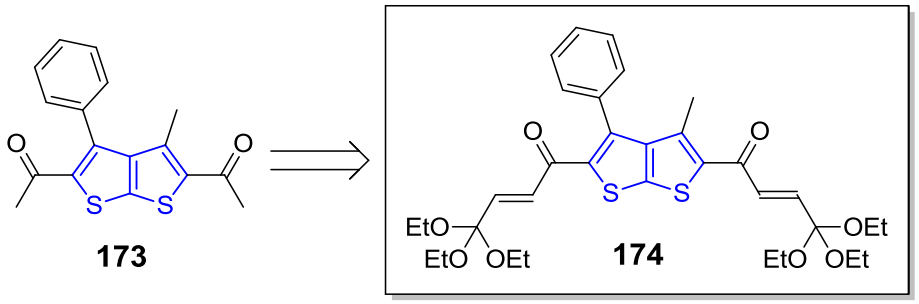


Fig. 55. Thienothiophene-based bacterial βGLU inhibitor.

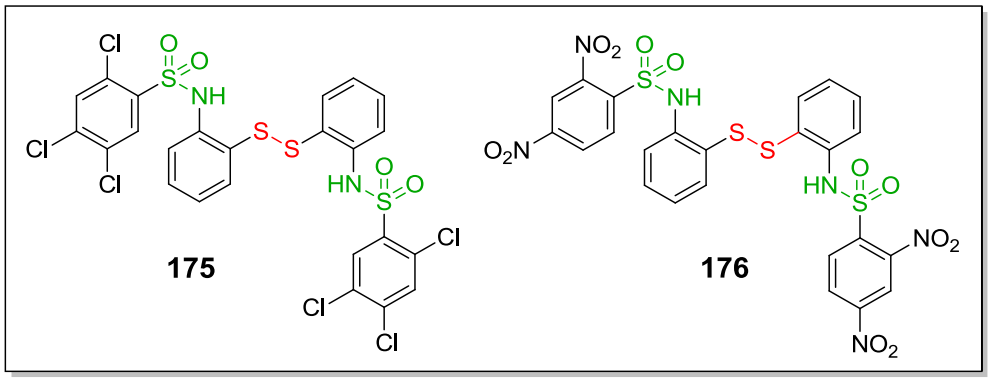


Fig. 56. Most active disulfide-benzenesulfonamide based βGLU inhibitor.

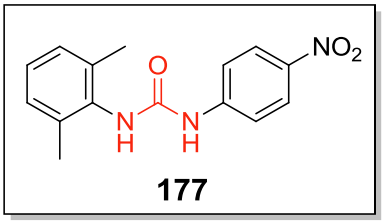


Fig. 57. Most potent urea based βGLU inhibitor.

compared to those with unsubstituted *N*-phenyl ring or NO_2 substituted ureas (Fig. 58). Akin to compound **141**, thioureas containing piperazine unit also exhibited improved potency compared to their morpholine and piperidine analogues, while *N*-alkylation of piperazine with *N*-(*o* or *p*-methoxyphenyl) or *N*-(2-pyridyl), abolished potency. Compounds **178** (IC_{50} = 0.86 μM) and **179** (IC_{50} = 1.24 μM) were the most potent inhibitors overall. In addition, 8-aminoquinoline derivatives **180** and **181** are also promising inhibitors with IC_{50} = 1.64 and 2.12 μM respectively.

3.2.8. Metal complexes and glycopolymers

Extant empirical data identified bacterial βGLU as a chelating agent, since enzyme activity was totally lost in the presence of Cu^{2+}, Hg^{2+} and Ag^{+} metal ions and restored in the presence of other chelating agents – Versene and cysteine [249]. Therefore, metal complexes with coordinatable metal centres exhibit significant bacterial βGLU inhibitory activities. To this end, Pd(II) complexes containing aniline and triphenylphosphine ligands were synthesized [250]. *Para*-chloroaniline derived metal complex **182** (Fig. 59), displayed the strongest activity against bacterial βGLU with 98.5% inhibition and IC_{50} = 15.40 μM. It was also 2- and 5-fold superior to *meta*-chloroaniline derived and *N*-methyl substituted metal complexes respectively. Similarly, *N*-heterocyclic carbene (NHC) complexes of Au(I) [251], as well as *bis* (NHC) complexes of Au(I) and Au(III) [252], containing hydroxy, chloride, acetoxy and acetonyl ligands were equally potent bacterial βGLU inhibitors with IC_{50} ranging from 0.14 to 2.60 μM. The strongest Au(I) NHC complex **183** (IC_{50} = 0.14 μM) was 327-fold more potent than reference D-SAL and 7-fold superior to strongest *bis* (NHC) complexes **184**. Nonetheless, undesirable cytotoxicity against 3T3 cells discomfits the therapeutic significance of these metal complexes.

Parallel to iminosugars **60**–**74**, the inhibitory potencies of structurally similar glycopolymers (Fig. 60) was dependent on the presence of carboxyl unit, type and configuration of sugar unit. Hence, glycopolymers with D-glucaric pendants linked to the polymer frame at C-1 (**185**), were stronger inhibitors compared to glycopolymers linked at C-5 (**186**) or those bearing D-gluconic (**187**) [253], D-mannaric (**188**) [254] and shorter chain D,L-xylaric (**189**) and L-tartaric (**190**) [255] pendants. However, these glycopolymers only show >60% inhibition at millimolar concentrations.

4. Patents describing the therapeutic significance and inhibition of βGLU activity

Patents and patent applications disclosing potent inhibition of βGLU particularly bacterial βGLU and the therapeutic significance thereof, have been documented. We therefore review in this section, patents applications filed in the present millennium relevant to βGLU inhibition. We have also included the only biomarker application of the enzyme filed within the period [256]. However, some of the patents and applications [257–263] emanated from published papers [19,210,228,229,241,262], reviewed in previous sections; therefore, only their summary is presented in Table 3.

Considering the limitations associated with traditional clinical assessments for periodontal diseases diagnosis and the improved

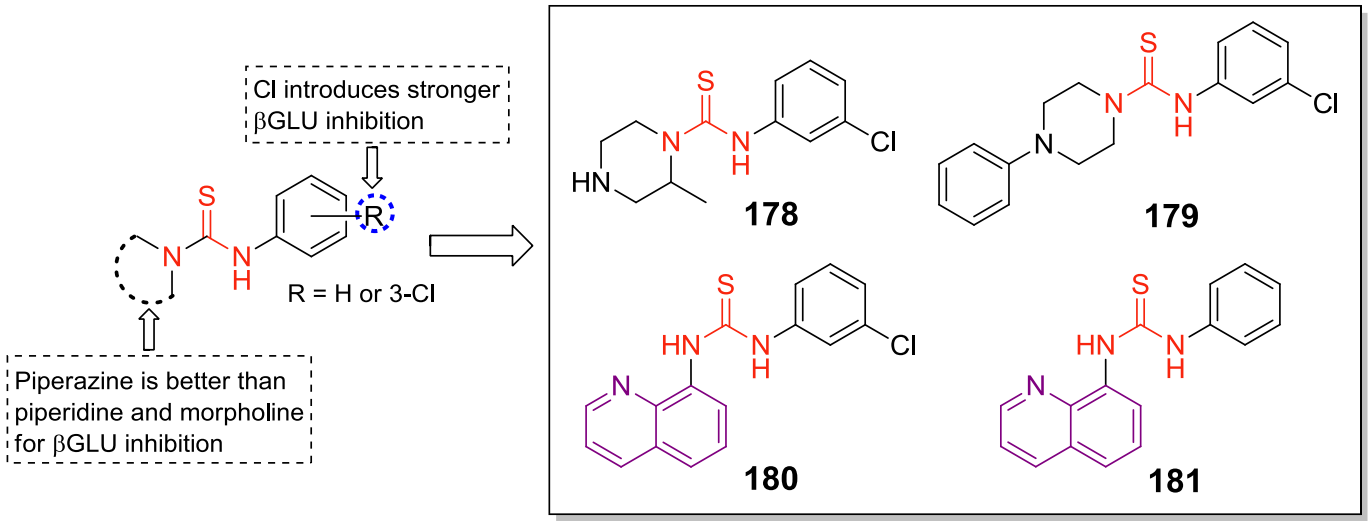


Fig. 58. SAR analysis of thioureas and most active βGLU inhibitors.

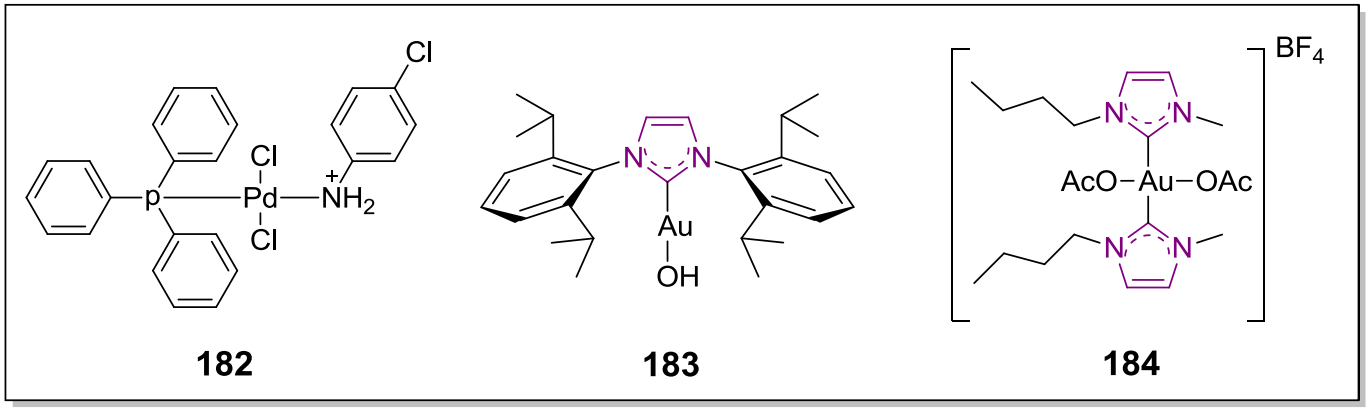


Fig. 59. Metal complexes for bacterial βGLU inhibition.

reliability of βGLU as a biomarker, a simple method requiring less expertise has been claimed to quantify elevated levels of the enzyme in the saliva of diseased patients relative to healthy ones, as a biomarker of disease risk and status [256]. The invention involves adding known β-D-glucuronide substrates (4-methylumbelliferone or phenolphthalein β-D-glucuronides) to a sample of patient's saliva in βGLU activity assay and thereafter quantifying the amount of aglycone produced *via* fluorometry, colorimetry or spectrophotometry. The addition of a labelled polyclonal or monoclonal antibody specific for βGLU to saliva sample and subsequent estimation of the amount of labelled antibody forming βGLU-antibody complex was also claimed, as well as a test-kit for claimed methods.

Bacterial βGLU-mediated hydrolysis of steroid glucuronides on the skin leads to body odours hence inhibitors of the process were developed and applied in deodorant and antiperspirant formulations [264]. The invention claimed that deodorant formulations with described compounds only inhibits βGLU activity without altering the natural composition of skin microbiota; in contrast to bacteriostatic or bactericidal mode of action of prior arts. Amongst the claimed inhibitors of different class, the strongest inhibition of *E. coli* βGLU was found with galactaric acid (99% inhibition at 0.1% test concentration in water), as well as zinc glycinate and zinc gluconate; 99% and 97% inhibition respectively at 0.25% test concentrations.

In the same vein, potent inhibitors of bacterial βGLU activity on urine have found non-therapeutic application in hygiene and sanitary products for suppressing the generation of urine odour [265]. At 0.1 wt% in dipropylene glycol, all the claimed inhibitors *viz.* macrocyclic ketones, ketones, macrocyclic lactones, macrocyclic oxalactones, esters, aldehydes, alcohols, ethers and terpenes, showed over 60% inhibition of *E. coli* βGLU. Macrocyclic ketones were stronger inhibitors than others. Consequently, the invention's most active inhibitor was compound **191** with 100%, 99.9% and 95.8% inhibition at 0.1, 0.01 and 0.001 wt% respectively. It was also effective after 48 h in suppressing the increase in urine odour when incorporated into different hygiene and sanitary products.

Phenoxy thiophene sulfonamides [266] were designed as adjuvants to eliminate the dose-limiting GI toxicity (diarrhoea) of CPT-11, through potent inhibition of bacterial βGLU-mediated deconjugation of active metabolite's glucuronide (SN-38G) in the intestine; thus, improving the drug's therapeutic efficacy. The synthesis of 76 βGLU inhibitors and 18 analogues of BRITE-355252 (compound **192**), the most active compound (IC_{50} = 20 nM), together with their therapeutic application was claimed. SAR study of compound **192** analogues revealed that inhibitory potency was markedly dependent on *N*-piperazinyl pendant at *meta*-position of phenoxy ring. Over 500-fold reduced inhibitory potency occurred with *N*-methylation (akin to compound **156**), 15-fold reduction

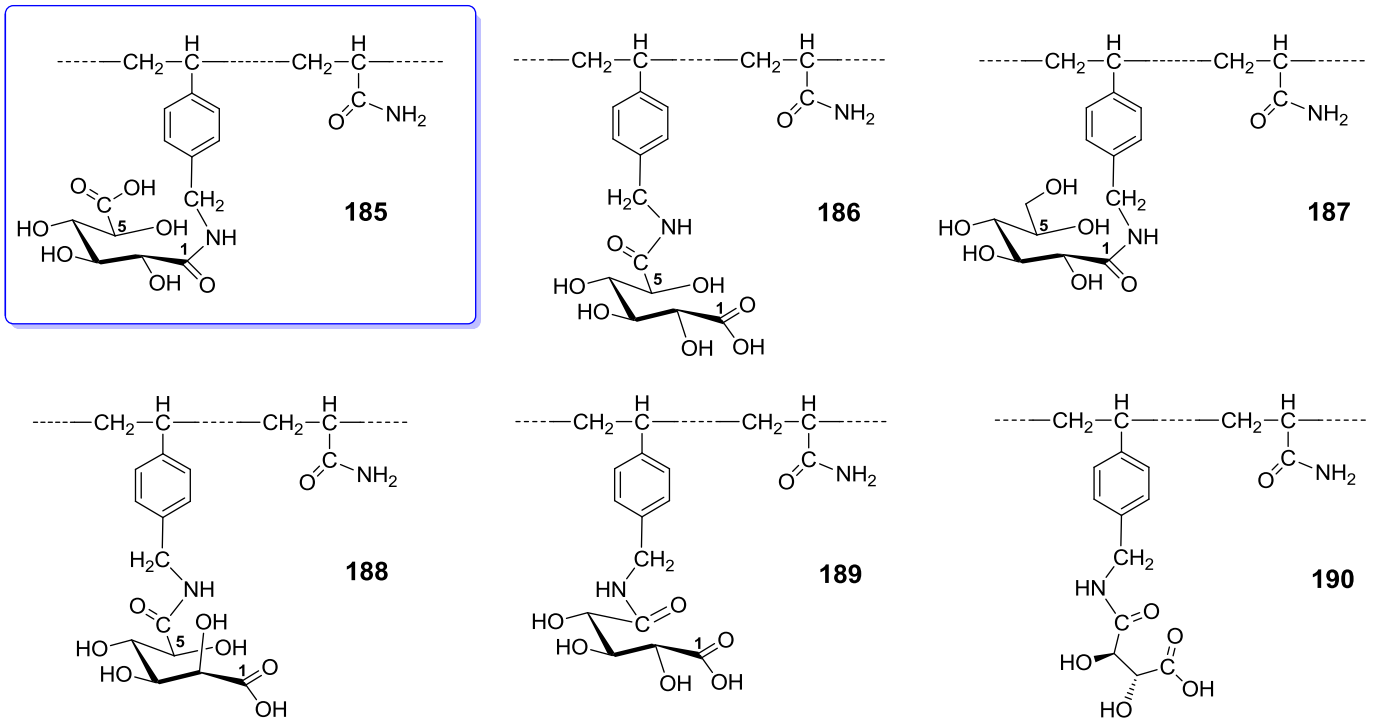


Fig. 60. Glycopolymer-based βGLU inhibitors and most active analogue (**185**) bearing pendant D-glucaric acid units.

when appended at *para*-position and total loss of activity when removed. Uninstalling the chloro unit on thiophene ring also slightly diminished potency by 5-fold. Whereas, replacing naphthyl with substituted phenyl units afforded inhibitors with similar potencies *i.e.* IC_{50} < 50 nM (Table SI); as a result, **193** was equipotent with **192**.

5. Concluding remarks

βGLU is a physiologically important lysosomal glycosyl hydrolase with appreciable therapeutic potentials such as biomarker for disease diagnosis, endogenous bioactivator in prodrug monotherapy and enzyme replacement therapy. The enzyme's role in cell proliferation and inflammation also renders it a potential target for anticancer and anti-inflammatory drugs development respectively. Moreover, since a significant number of commercially available drugs are metabolised by glucuronidation, hydrolytic activity (*i.e.* deglucuronidation) by βGLU in human intestinal microbiota has been linked to colon carcinogenesis and genotoxicity as well as drug-induced dose-limiting toxicities of anticancer agents and NSAIDs, which thwarts their therapeutic potential and clinical utility. Therefore, potent inhibition of βGLU holds enormous clinical importance.

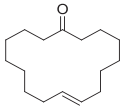
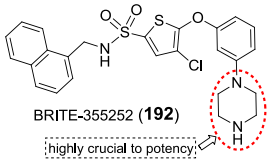
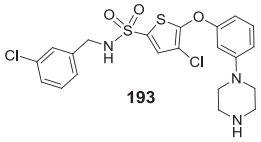
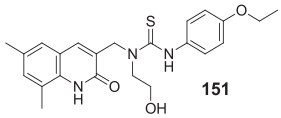
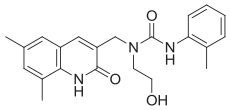
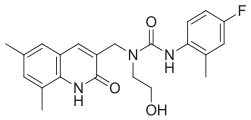
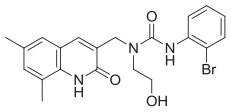
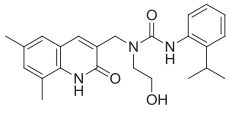
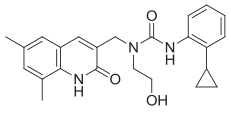
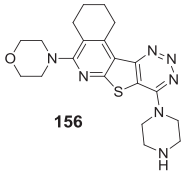
Generally, our literature survey found that a significant number of natural products-derived inhibitors display moderate inhibition of βGLU, and increased potency is found with inhibitors containing a flavonoid skeleton. The physiological tolerance, acceptable toxicity and favourable pharmacodynamic profiles of these natural inhibitors, posits them as worthy scaffolds warranting witty molecular developments. In addition, iminosugars distinguish themselves as a unique class of natural product-inspired molecules with promising potentials regardless of their usually multiple synthetic steps. Notable amongst these are the nojirimycin analogues and iminosugar *C*-glycosides, both conferred with highly selective inhibition of bacterial βGLU. However, the inhibitory potency of these carbohydrate mimics relies on several factors such as sugar configuration, mimicry of glucuronic acid substrate and correct lipophilic balance. Hence, to harness their remarkable potential, ingenious manipulations of the synthetic medicinal chemist interested in exploring their chemical space is expressly required.

On the other hand, purely synthetic inhibitors show fascinating diversity in their inhibitory potencies and pattern, due to the plethora of pharmacophoric enrichments possible for a single molecular design. Based on the foregoing, a comprehensive list of potent synthetic inhibitors ($IC_{50} \leq 5$ μM) in this review is provided in Table SI of supporting information. Evinced by their nanomolar IC_{50} values, quinoline-pyrazoles (**167** and **168**), piperazine-containing compounds (**156** and **157**; **192** and analogues) as well as the indole nucleus, are conferred with the strongest inhibition of βGLU activity overall. The order of potency for hydrazone tethers was found as; *bis*indole ˃ indole > quinoline > thiazole ˃ benzothiazole ≈ oxadiazole. Whereas, NO_2, Cl, particularly F and OH substituents at *ortho*- or *para*-positions usually produce stronger βGLU inhibitors compared to their *meta*-substitutions.

Taken together, these suggests that the presence of polar groups with strong hydrogen bonding potential is crucial to inhibitory potency, while an indiscriminate increase in molecular lipophilicity is detrimental to strong βGLU inhibition. Consequently, we envisage that the model βGLU inhibitor will be that which is appositely tuned in its lipophilicity and polarity for easy cell penetration, favourable fit into the binding/catalytic pocket of βGLU for energetically stable binding and strong enzyme inhibition. We also perceive that rationale molecular hybridization of those active class of natural products and synthetic molecules will furnish stronger inhibitors of βGLU with improved selectivity and acceptable toxicity profile.

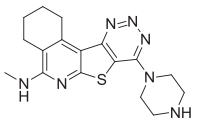
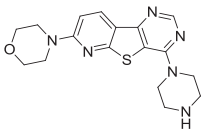
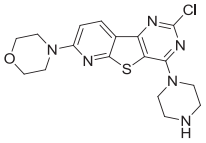
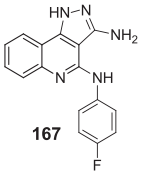
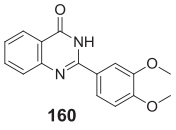
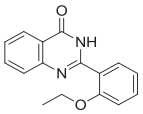
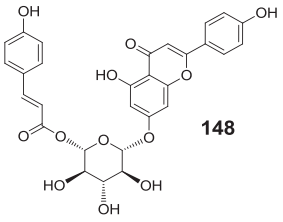
Furthermore, we found that due to the mixture of electroneutral and electronegative spots on the surface of bacterial βGLU binding pocket at physiological pH, in contrast to the electropositive spots for human βGLU, the presence of a protonatable *N*-atom confers

Table 3
Filed patents (2000–2019) describing the therapeutic significance of βGLU activity and its inhibition.

Patent Number	Invention	Most active βGLU inhibitor	IC_{50} (nM)	Therapeutic significance
US 6,277,587 B1	Simple method for quantifying the elevated levels of βGLU in saliva sample of periodontally diseased patients by adding glucuronide substrates or labelled βGLU-selective antibody	–	–	Biomarker of periodontal disease risk and status
US 7,294,330 B2	Deodorant and antiperspirant formulations containing compounds which inhibits odour-producing skin bacterial βGLU-mediated hydrolysis of steroid glucuronides, without affecting skin microbiota composition	• Galactaric acid • Zinc glycinate • Zinc gluconate	99%[a] 99%[a] 97%[a]	Non-therapeutic cosmetic formulations
US 9.200,269 B2	Incorporating potent inhibitors into hygiene and sanitary products to suppress the generation of urine or other excreta odour due to bacterial βGLU activity on the body wastes	 **191**	99%[a]	Non-therapeutic products to suppress urine odour
US 9617.239 B2	Phenoxy thiophene sulfonamides as CPT-11 co-drugs for potent and selective inhibition of intestinal bacterial βGLU activity	 BRITE-355252 (**192**) highly crucial to potency  **193**	20	Alleviation of drug induced dose-limiting toxicities to improve therapeutic efficacy
US 2015/0011542 A1	Safe and effective methods for alleviating NSAID-induced GI tract injuries *via* therapeutic doses of potent and selective bacterial βGLU inhibitors	 **151**	140	
WO 2019/051185 Al	Compounds, compositions and methods for selective inhibition of intestinal bacterial βGLU to reduce the side effects (diarrhoea) of camptothecin-derived antineoplastic agents or other drugs or metabolites or compounds that are βGLU substrates, used in treating neoplasms or other conditions.		118	
			142	
			347	
			468	
			759	
US 9334.288 B2		 **156**	1.17	
WO 2018/017874 Al			*Ec* = 70 *Sa* = 39 *Cp* = 180	

(*continued on next page*)

Table 3 (*continued*)

Patent Number	Invention	Most active βGLU inhibitor	IC_{50} (nM)	Therapeutic significance
				
			*Ec* = 130 *Sa* = 690 *Cp* = 53	
			*Ec* = 30 *Sa* = NA *Cp* = NA	• Food supplements to prevent carcinogen induced colon carcinoma
US 2018/0214426 A1	Methods for preparing pyrazolo[4,3-*c*]quinoline derivatives for selective inhibition of microbiota βGLU and their therapeutic applications as; (1) chemotherapy adjuvants to reduce chemotherapy-induced diarrhoea and enhance chemotherapy efficiency (2) health-food supplements to prevent colon carcinoma	 **167**	120	
US 2014/0256754 Al	Preparation of quinazolin-4(3*H*)-ones-based βGLU inhibitors	 **160**	600	Therapeutic intervention for health disorders stimulated by high βGLU activity
		 **161**	700	
US 2017/0009272 A1	Virtual screening methods for identifying potent inhibitors of βGLU	 **148**	450	450

[a] % inhibition at test concentration; *Ec* = *E. coli* βGLU; *Sa* = *S. agalactiae* βGLU; *Cp* = *C. perfringens* βGLU; NA - not active.

improved selectivity for bacterial βGLU inhibition. This was particularly true for piperazine based inhibitors. Therefore, we believe further probing of this phenomenon will allow structure-activity guided molecular tuning to afford stronger βGLU inhibitors.

To the best of our knowledge, mechanistic studies on the behaviour of these inhibitors is scanty in literature and only found in two articles describing *gluco*-imidazole (**60**) and piperazine (**156**) based inhibitors. This investigative approach provides insight to the behaviour of these inhibitors in the catalytic cycle of βGLU thereby allowing deeper understanding of important structural features crucial to inhibitory activity beyond SAR and molecular docking studies. Finally, we hope this review will inspire medicinal chemists to direct their search light towards this molecular target, for the development of new therapeutic agents with improved efficacy and betterment of human health in consequential.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.111921.

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drug design: sugar amino acids and carbohydrate scaffolds, Chem. Rev. 102 (2002) 491–514, https://doi.org/10.1021/cr0004409.
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List of abbreviations

AChE: Acetylcholinesterase
AcP: Acid phosphatase
ADP: Adenosine diphosphate
ALT: Alanine aminotransferase
ABL: Alveolar bone loss
AST: Aspartate aminotransferase
BOP: Bleeding on probing
βGLU: beta-glucuronidase
BALF: Bronchoalveolar lavage fluid
BuChE: Butyrylcholinesterase
CPT-11: Irinotecan – an analogue of Camptothecin
CSF: Cerebrospinal fluid
CAL: Clinical attachment level
DMBA: 7,12-dimethylbenz[α]anthracene
DMH: 1,2-dimethylhydrazine
D-SAL: D-saccharic acid-1,4-lactone
D-SDL: 2,5-di-*O*-acetyl-D-glucaro-1,4:6,3-dilactone
D-GL: D-glucurono-γ-lactone
GCF: Gingival crevicular fluid
GH: Glycosyl hydrolase
GI: Gastrointestinal
Gg-I: Gingival index
GPCR: G protein-coupled receptors
H-b: Hydrogen bonding
IC_{50}: Half maximal inhibitory concentration
IL-1β: Interleukin-1 beta
IL-8: Interleukin-8
k_{cat}: Rate constant of catalysed reaction
kDa: kilodalton
K_i: Inhibition constant
k_{uncat}: Rate constant of uncatalyzed reaction
LAB: Lactic acid bacteria
LDH: Lactic acid dehydrogenase
MERS-CoV: Middle East respiratory syndrome coronavirus
MMPs: Matrix metalloproteinases
NSAID: Nonsteroidal anti-inflammatory drug
OP: Organophosphorus compounds
PARP: Poly (ADP-ribose) polymerase
PD: Probing depth
SAR: Structure-activity relationship
S_N2: Nucleophilic substitution 2
SN-38: Active metabolite of CPT-11
SN-38G: Glucuronide conjugate of SN-38
TNF-α: Tumour necrosis factor-α
UDP: Uridine-5′-diphosphate
UGT: UDP-glucuronosyltransferase