Phenoxyacetohydrazide Schiff Bases: β-Glucuronidase Inhibitors

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Abstract: Phenoxyacetohydrazide Schiff base analogs 1–28 have been synthesized and their in vitro β-glucuronidase inhibition potential studied. Compounds 1 (IC50 = 9.20 ± 0.32 µM), 5 (IC50 = 9.47 ± 0.16 µM), 7 (IC50 = 14.7 ± 0.19 µM), 8 (IC50 = 15.4 ± 1.56 µM), 11 (IC50 = 19.6 ± 0.62 µM), 12 (IC50 = 30.7 ± 1.49 µM), 15 (IC50 = 12.0 ± 0.16 µM), 21 (IC50 = 13.7 ± 0.40 µM) and 22 (IC50 = 22.0 ± 0.14 µM) showed promising β-glucuronidase inhibition activity, better than the standard (D-saccharic acid-1,4-lactone, IC50 = 48.4 ± 1.25 µM).
Keywords: phenoxyacetohydrazide; Schiff bases; β-glucuronidase; glucuronosyl-O-bonds, D-saccharic acid-1,4-lactone

1. Introduction

A range of bioactivities are reported for hydrazide-hydrazone compounds, such as antibacterial, anticonvulsant, antimalarial, analgesic, antiinflammatory, antiplatelets, antifungal, antituberculosis, and anticancer activities [1–6]. A variety of semicarbazones, thiosemicarbazones and guanyl hydrazones are found to be key compounds for drug design [7], for metal complexes [8], organocatalysis [9], and are used for the preparation of heterocyclic rings [10]. A few pyrazole carboxyamide hydrazide hydrazones were found to be proliferation inhibitors of A549 cells [12,13] Some evidence proposes a pharmacophoric character for the hydrazone moiety present in phenylhydrazine derivatives in the inhibition of cyclooxygenase [14]. Antioxidant [15–18], antiglycation [19–22] and antileishmanial [23] activity have recently been reported, as well as applications in mass spectrometry [24].

The present work aimed to investigate the potential activity of a series of aryl hydrazide-hydrazones as in vitro β-glucuronidase inhibitors. In our designed analogues substituted phenoxy-acetohydrazides were treated with different aromatic aldehydes to scrutinize their potential activity. The earlier reported literature [25] showed that β-glucuronidase is a lysosomal enzyme, present in many organs like the spleen, kidney, lung, bile, serum and urine, etc., where its specific task is to catalyze the cleavage of glucuronosyl-O-bonds [26–28]. It degrades glucuronic acid-containing glycosaminoglycans, like heparan sulfate, chondroitin sulfate and dermatan sulfate [29]. An elevated level of β-glucuronidase was observed in various types of malignancies, such as breast, lung and gastrointestinal tract carcinomas, and melanomas. Its high expression also observed in bronchial tumors [30]. On the other hand, mucopolysaccharidosis type VII (MPS VII; Sly Syndrome) is caused by the deficiency of human β-glucuronidase [31]. The circulating level of β-glucuronidase is also useful as a lysosomal enzyme in children affected by leprosy. In borderline tuberculoid patients and lepromatous patients higher activity of this enzyme was also observed.

2. Results and Discussion

2.1. Chemistry

Lead identification is a well defined tool in drug design and discovery. Our research group has been involved for a decade in lead discovery programs in search of novel therapeutic agents. We have earlier reported Schiff bases of different classes of organic compounds in the search for lead molecules with different biological activities [32–34]. Earlier, our group reported the leishmanicidal and β-glucuronidase inhibition potential of hydrazides derived from the corresponding esters [35–38]. In view of the formerly reported work we synthesized hydrazide Schiff bases and screened their potential biological activities [39–42]. Acylhydrazide Schiff base derivatives 1–28 were synthesized from an acylhyrazide by condensing it with different aromatic aldehydes and acetophenones under reflux
conditions in ethanol for 2–3 h (Scheme 1). The crude products (Table 1) were further recrystallized
from methanol and needle-like crystals were obtained in most of the cases. The starting acylhydrazide was
synthesized from ester of ethyl 2-(4-chloro-2-methylphenoxy) acetate by refluxing with hydrazine hydrate.

**Scheme 1.** Synthetic scheme for benzohydrazide followed by synthesis of Schiff bases 1–28.

**Table 1.** Synthesis of acylhydrazide Schiff base derivatives 1–28.

| Compound No. | R<sub>1</sub>     | R<sub>2</sub> | Yield (%) | Compound No. | R<sub>1</sub> | R<sub>2</sub> | Yield (%) |
|--------------|------------------|--------------|-----------|--------------|----------------|--------------|-----------|
| 1            | 6'               | H            | 81        | 15           | 5'            | H            | 88        |
|              | 5'               | OMe          |           |              |                |              |           |
|              | 2'               |              |           |              |                |              |           |
| 2            | 6'               | H            | 85        | 16           | 5'            | H            | 92        |
|              | 5'               | OMe          |           |              |                |              |           |
|              | 3'               |              |           |              |                |              |           |
| 3            | 6'               | H            | 93        | 17           | 5'            | CH<sub>3</sub> | 94        |
|              | 5'               |              |           |              |                |              |           |
|              | 3'               |              |           |              |                |              |           |
| 4            | 6'               | H            | 87        | 18           | 5'            | H            | 89        |
|              | 5'               | OMe          |           |              |                |              |           |
|              | 2'               | MeO          |           |              |                |              |           |
|              | 3'               | OMe          |           |              |                |              |           |
| Compound No. | R_1 | R_2 | Yield (%) | Compound No. | R_1 | R_2 | Yield (%) |
|--------------|-----|-----|-----------|--------------|-----|-----|-----------|
| 5            |     | H   | 83        | 19           |     | CH₃ | 87        |
| 6            |     | H   | 86        | 20           |     | H   | 84        |
| 7            |     | H   | 89        | 21           |     | H   | 94        |
| 8            |     | H   | 91        | 22           |     | H   | 93        |
| 9            |     | H   | 94        | 23           |     | H   | 91        |
| 10           |     | H   | 82        | 24           |     | CH₃ | 95        |
| 11           |     | H   | 88        | 25           |     | H   | 91        |
| 12           |     | H   | 91        | 26           |     | H   | 88        |
| 13           |     | H   | 86        | 27           |     | H   | 81        |
| 14           | Me  | H   | 93        | 28           |     | H   | 93        |
2.2. β-Glucuronidase

Synthetic acyl hydrazides Schiff bases 1–28 were screened for their in vitro potential as β-glucuronidase inhibitors. The in vitro β-glucuronidase inhibitory potential was evaluated by using the literature protocol [43]. Compounds 1–28 showed diversified β-glucuronidase inhibitory activities, with IC₅₀ values ranging between 9.20–30.7 µM. Compounds 1, 5, 7, 8, 11, 12, 15, 21, and 22 showed excellent β-glucuronidase inhibitory activities, with IC₅₀ values of 9.20 ± 0.32, 9.47 ± 0.16, 14.7 ± 0.19, 15.4 ± 1.56, 19. ± 0.62, 30.7 ± 1.49, 12.0 ± 0.16, 13.7 ± 0.40, and 22.0 ± 0.14 µM, respectively, and the remaining compounds exhibited no activity (Table 2).

Table 2. In vitro β-glucuronidase activity of compounds 1–28.

| Compounds | IC₅₀ (µM ± SEM a) | Compounds | IC₅₀ (µM ± SEM a) |
|-----------|-------------------|-----------|-------------------|
| 1         | 9.20 ± 0.32       | 15        | 12.0 ± 0.16       |
| 2         | NA b              | 16        | NA b              |
| 3         | NA b              | 17        | NA b              |
| 4         | NA b              | 18        | NA b              |
| 5         | 9.47 ± 0.16       | 19        | NA b              |
| 6         | NA b              | 20        | NA b              |
| 7         | 14.7 ± 0.19       | 21        | 13.7 ± 0.40       |
| 8         | 15.4 ± 1.56       | 22        | 22.0 ± 0.14       |
| 9         | NA b              | 23        | NA b              |
| 10        | NA b              | 24        | NA b              |
| 11        | 19.6 ± 0.62       | 25        | NA b              |
| 12        | 30.7 ± 1.49       | 26        | NA b              |
| 13        | NA b              | 27        | NA b              |
| 14        | NA b              | 28        | NA b              |
| D-saccharic acid-1,4-lactone c | 48.4 ± 1.25 | - | - |

SEM a is the standard error of the mean; NA b Not active; c standard inhibitor for β-glucuronidase.

It was observed that both the substituents’ nature and their position at the benzilidine part have great importance in the β-glucuronidase inhibition activity of a compound, and apparently the acylium part does not take part in the activity (Figure 1).

Figure 1. The two parts of molecule on which activity is based.

The best activity was shown by compound 1 (IC₅₀ = 9.20 ± 0.32 µM, fivefold better than the standard D-saccharic acid-1,4-lactone, IC₅₀ value 48.4 ± 1.25 µM) which has a methoxy group at the ortho position. Surprisingly, a marked decline in activity (to the point of being inactive) was observed
in analog 2 which has a methoxy residue at the para position instead of the ortho position as in compound 1. This huge difference in the activities of compounds 1 and 2 clearly indicates that a specific group at a specific position of the benzylidine phenyl ring part plays a vital role in making a potent β-glucuronidase inhibitor in this type of compounds. The excellent activity of compound 5 (IC\textsubscript{50} = 9.47 ± 0.16 µM) having an ortho nitro group on the phenyl ring as compared to the inactivity of its closely related derivative 6 having a meta nitro group on phenyl ring proves our hypothesis that a suitable group at a suitable position of the phenyl ring of benzilidine part of molecules is a prerequisite for β-glucuronidase inhibitory potential in these N-acylhydrazone Schiff bases. Comparison of activity of chloro-containing compounds 7 (IC\textsubscript{50} = 14.7 ± 0.19 µM), 8 (IC\textsubscript{50} = 15.4 ± 1.56 µM), and 9 (inactive) demonstrated that the nature and location of a substitution is important for β-glucuronidase inhibitory potential. Dichloro-substituted compounds 9 and 10 were found to be completely inactive which further proves our hypothesis. Compound 11 (IC\textsubscript{50} = 19.6 ± 0.62 µM) having an ortho fluoro group showed excellent activity, but a little less than analogous chloro compounds 7 and 8. We also evaluated the effect of heterocyclic ring-containing derivatives, and it was observed that the five membered heterocyclic thiophene ring-containing derivative 12 (IC\textsubscript{50} 30.7 ± 1.49 µM) produced remarkable activity, while on the other hand five membered heterocyclic rings like furan and its methyl derivatives 13 and 14 were found to be completely inactive. Almost all mono-, di- and trihydroxy substituted compounds 16, 17, 18, 19, and 20 found to be completely inactive, but unexpectedly compound 15 (IC\textsubscript{50} = 12.0 ± 0.16 µM) which bears 2,3-dihydroxy substitution, was found to be very efficient and displayed remarkable activity, better than the standard, but, compound 16, also a 3,4-dihydroxy derivative did not show any activity. N-acylhydrazones Schiff base 21 (IC\textsubscript{50} = 13.7 ± 0.40 µM) synthesized from 1-napthaldehyde was found to be more active than 22 (IC\textsubscript{50} = 22.0 ± 0.14 µM) which was synthesized from 2-napthaldehyde, both without any substitution. Remaining compounds 23–28 were found to be completely inactive. This pattern of activity reveals that the substituent and its position on the phenyl ring of benzylidine part is a driving force for β-glucuronidase inhibition activity.

In conclusion, a number of potential lead molecules has been identified as β-glucuronidase inhibitors. Compounds 1, 5, 7, 8, 11, 12, 15, 21, and 22 demonstrated excellent activity and it is anticipated that by slight synthetic modification in these molecules, some new most active β-glucuronidase inhibitors can be developed.

3. Experimental

3.1. General Information

\(^1\)H-NMR experiments were performed on an Avance-Bruker AM 300 MHz instrument (Wissembourg Cedex, France). A Carlo Erba Strumentazione-Mod-1106 (Milan, Italy) used to measure CHN analysis. EI MS was performed on a Finnigan MAT-311A (Bremen, Germany). Thin layer chromatography (TLC) was carried out on pre-coated silica gel glass plates (Kieselgel 60, 254, E. Merck, Darmstadt, Germany). The chromatograms were visualized by UV at 254 and 365 nm or iodine vapours.
3.2. Biological Assays

β-Glucuronidase (E.C. 3.2.1.31 from bovine liver, G-0251) and p-nitrophenyl-β-D-glucuronide (N-1627) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Anhydrous Na₂CO₃ and all other reagents of standard grade were obtained from E. Merck. The anhydrous EtOH and CHCl₃ used in the experiments were dried employing the standard methods. All other solvents and reagents like the benzoil chloride were of standard grade.

3.3. Assay for β-D-Glucuronidase

β-D-Glucuronidase inhibition was determined by measuring the absorbance of the p-nitrophenol which is produce from the substrate at 405 nm. The total reaction volume was 250 µL. The reaction mixture contains 5 µL of test compound solution, 185 µL of 0.1 M acetate buffer, and 10 µL of enzyme, and it was incubated at 37 °C for 30 min. The plates were read on a multiplate reader at 405 nm after the addition of 50 µL of 0.4 mM p-nitrophenyl-β-D-glucuronide. All assays were performed in triplicate.

3.4. Typical Method for the Synthesis of Compounds 1–28

To a mixture of 2-(4-chloro-2-methylphenoxyacetic acid) hydrazide (1 mmol) in methanol (25 mL) was added a substituted aldehyde (1 mmol) and 3 drops of glacial acetic acid and the mixture was refluxed for 3 h. After completion of the reaction (TLC analysis), it was cooled and evaporated on a rotary evaporator. The resultant crude product was crystallized from methanol to afford 80%–90% yields of pure product. The structures of synthetic compounds 1–28 were determined by different spectroscopic techniques, including ¹H-NMR, and EI MS spectroscopy.

2-(4-Chloro-2-methylphenoxy)-N’-[3-(2-methoxyphenyl)-2-propenylidene]acetohydrazide (1). Yield: 68%; ¹H-NMR (DMSO-d₆) δ 8.04 (d, 1H, J = 9.3 Hz, N=CH-CH), 7.63 (dd, 1H, Jₛ,₆ = 7.8 Hz, Jₛ,₃ = 1.2 Hz, H-6’), 7.34 (dd, 1H, J₇CH-H₃=CH = 8.1 Hz, CH-H₃=CH), 7.23 (d, 1H, J₇HC=CH = 6 Hz, H₇=CH), 7.12–7.20 (m, 2H, H-3/5) 6.85–7.06 (m, 3H, H₃/4'/5’), 6.80 (d, 1H, J₆,₅ = 9 Hz, H-6), 5.17 (s, 2H, -OCH₂), 3.84 (s, 3H, -OCH₃), 2.23 (s, 3H, CH₃); EI MS: m/z (%) 358 (M⁺, 30), 327 (100), 189 (40), 175 (55), 159 (100), 125 (75.0), Anal. Calcd for C₁₉H₁₉ClN₂O₃, C = 63.60, H = 5.34, N = 7.81. Found: C = 63.55, H = 5.31, N = 7.80.

2-(4-Chloro-2-methylphenoxy)-N’-[3-(4-methoxyphenyl)-2-propenylidene]acetohydrazide (2). Yield: 70%; ¹H-NMR (DMSO-d₆) δ 8.02 (d, 1H, J = 9.3Hz, N=CH-CH), 7.55 (d, 2H, J₇,₂ = J₆,₅ = 8.7 Hz, H₂/6’),7.12–7.23 (m, 2H, H-3/5),7.01 (d, 2H, J₅,₂ = J₅,₆ = 7.0 Hz, H₃/5’), 6.76–6.87 (m, 2H, HC=CH), 6.92 (s, 2H, -OCH₂), 3.84 (s, 3H, -OCH₃), 2.23 (s, 3H, CH₃); EI MS: m/z (%) 358 (M⁺, 95), 189 (40), 175 (85), 159 (100), 125 (60). Anal. Calcd for C₁₉H₁₉ClN₂O₃, C = 63.60, H = 5.34, N = 7.81. Found: C = 63.57, H = 5.31, N = 7.80.

2-(4-Chloro-2-methylphenoxy)-N’-[4-(4-ethoxyphenyl)methylidene]acetohydrazide (3). Yield: 80%; ¹H-NMR (DMSO-d₆) δ 8.20 (s, 1H, -N=CH), 7.62 (d, 2H, J₆,₅ = 8.7 Hz, H-2/6’), 7.23–7.12 (m, 2H, H-3/5), 6.84 (d, 2H, J₃,₂ = J₅,₆ = 8.7 Hz, H₃/5’), 6.84 (d, 1H, J₆,₅ = 8.7 Hz, H-6), 5.13 (s, 2H, OCH₂), 4.04 (q,
2H, J = 6.9 Hz, -CH2), 2.22 (s, 3H, -CH3), 1.32 (t, 3H, J = 6.9 Hz, -CH3); El MS: m/z (%): 347 (M+, 56), 205 (95), 155 (89), 147 (92), 119 (73). Anal. Calcd for C18H19ClN2O3, C = 62.34, H = 5.52, N = 8.08. Found: C = 62.33, H = 5.50, N = 8.03.

2-(4-Chloro-2-methylphenoxy)-N'-[(3,4,5-trimethoxyphenyl)methylidene]acetohydrazide (4). Yield: 72%; 1H-NMR (DMSO-d6) δ 8.43 (s, 1H, -N=CH), 7.56 (d, 1H, J2,6 = 2.7 Hz, H-2'), 7.23 (m, 1H, H-5), 7.13 (d, 1H, J3,5 = 2.7 Hz, H-3), 6.92 (s, 1H, H-6'), 6.83 (d, 1H, J5,6 = 8.7 Hz, H-6), 5.13 (s, 2H, -OCH2), 3.82 (s, 9H, -OCH3), 2.22 (s, 3H, -CH3); EI MS: m/z (%): 392 (M+, 62.1), 251 (15.5), 193 (100), 179 (88.9), 155 (26.8). Anal. Calcd for C19H21ClN2O5, C = 58.09, H = 5.39, N = 7.13. Found: C = 58.05, H = 5.37, N = 7.11.

2-(4-Chloro-2-methylphenoxy)-N'-[(2-nitrophenyl)methylidene]acetohydrazide (5). Yield: 65%; 1H-NMR (DMSO-d6) δ 8.09 (s, 1H, -N=CH), 8.29 (m, 2H, H-4'/5'), 7.98 (d, 2H, J3',4' = J6',5' = 9, H-3'/6'), 7.24 (m, 1H, H-5), 7.13 (d, 1H, J3,5 = 2.7 Hz, H-3), 6.89 (d, 1H, J6,5 = 8.7 Hz, H-6), 5.22 (s, 2H, -OCH2), 2.22 (s, 3H, -CH3); EI MS: m/z (%): 347 (M+, 72), 206 (100), 155 (83.3), 125 (75.8). Anal. Calcd for C16H14ClN3O4, C = 55.26, H = 4.06, N = 12.08. Found: C = 55.23, H = 4.02, N = 12.04.

2-(4-Chloro-2-methylphenoxy)-N'-[(3-nitrophenyl)methylidene]acetohydrazide (6). Yield: 70%; 1H-NMR (DMSO-d6) δ 8.40 (s, 1H, -N=CH), 8.51 (d, 1H, J5',6' = 7.2 Hz, H-6'), 8.12–8.25 (m, 2H, H-2'/5'), 7.76 (m, 1H, H-4'), 7.12–7.23 (m, 2H, H-3/5), 6.90 (dd, 1H, J6,5 = 6.2 Hz, H-6), 5.22 (s, 2H, -OCH2), 2.19 (s, 3H, -CH3); EI MS: m/z (%): 347 (M+, 92), 206 (100), 178 (28.4), 141 (17.5), 125 (39.9). Anal. Calcd for C19H21ClN2O5, C = 58.09, H = 5.39, N = 7.13. Found: C = 58.06, H = 5.37, N = 7.12.

2-(4-Chloro-2-methylphenoxy)-N'-[(4-chlorophenyl)methylidene]acetohydrazide (7). Yield: 63%; 1H-NMR (DMSO-d6) δ 8.27 (s, 1H, -N=CH), 7.73 (d, 2H, J2,3 = J6,5 = 8.7 Hz, H-2'/5'), 7.51 (d, 2H, J2',3' = J6',5' = 8.1 Hz, H-3'/6'), 7.13–7.23 (m, 2H, H-3/5), 6.89 (d, 1H, J6,5 = 7.8 Hz, H-6), 5.15 (s, 2H, -OCH2), 2.19 (s, 3H, -CH3); EI MS: m/z (%): 336 (M+, 20), 195 (35), 155 (55), 125 (80), 89 (100). Anal. Calcd for C16H14Cl2N2O2, C = 56.99, H = 4.18, N = 8.31. Found: C = 56.95, H = 4.15, N = 8.28.

2-(4-Chloro-2-methylphenoxy)-N'-[(2-chlorophenyl)methylidene]acetohydrazide (8). Yield: 66%; 1H-NMR (DMSO-d6) δ 8.26 (s, 1H, -N=CH), 7.77 (m, 2H, H-3'/6'), 7.48 (br.t, 2H, J4,5 = J5,4 = 8.5 Hz, H-4'/5'), 7.13–7.24 (m, 1H, H-5), 6.89 (d, 1H, J6,5 = 8.7 Hz, H-6), 5.15 (s, 2H, -OCH2), 2.23 (s, 3H, -CH3), 2.19 (s, 3H, -CH3); EI MS: m/z (%): 336 (M+, 10), 195 (30), 155 (40), 125 (75), 89 (100). Anal. Calcd for C16H14Cl2N2O2, C = 56.99, H = 4.18, N = 8.31. Found: C = 56.96, H = 4.15, N = 8.27.

2-(4-Chloro-2-methylphenoxy)-N'-[(3,4-dichlorophenyl)methylidene]acetohydrazide (9). Yield: 70%; 1H-NMR (DMSO-d6) δ 8.25 (s, 1H, -N=CH), 7.55 (s, 1H, H-2'), 7.68 (br. s, 2H, 5'/6'), 7.12–7.23 (m, 2H, H-3/5), 6.88 (d, 1H, J6,5 = 7.0 Hz, H-6), 5.08 (s, 2H, OCH2), 2.22 (s, 3H, CH3); EI MS: m/z (%): 371 (M+, 92.2), 228 (100), 155 (87.1), 122 (89.8). Anal. Calcd for C16H13Cl2N2O2, C = 51.71, H = 3.53, N = 7.54. Found: C = 51.68, H = 3.50, N = 7.52.

2-(4-Chloro-2-methylphenoxy)-N'-[(2, 6-dichlorophenyl)methylidene]acetohydrazide (10). Yield: 60%; 1H-NMR (DMSO-d6) δ 8.48 (s, 1H, -N=CH), 7.57 (d, 2H, J3,4 = J5,4 = 8.7 Hz, H-3'/5'), 7.44 (dd, 1H, J4,5 = 7.2 Hz, H-4'), 7.24–7.11 (m, 2H, H-3/5), 6.78 (d, 1H, J6,5 = 8.7 Hz, H-6), 5.09 (s, 2H, -OCH2), 2.18
(s, 3H, CH3); EI MS: m/z (%) 370 (M+, 17.3), 229 (39.5), 155 (60.1), 125 (100), 89 (62). Anal. Calcd for C16H13Cl3N2O2, C = 51.71, H = 3.53, N = 7.54. Found: C = 51.66, H = 3.51, N = 7.52.

2-(4-Chloro-2-methylphenoxy)-N'-[(2-fluorophenyl)methylidene]acetohydrazide (11). Yield: 74%; 1H-NMR (DMSO-d6) δ 8.52 (s, 1H, -N=CH), 7.95 (dd, 1H, J4',5' = 8.1 Hz, H-5'), 7.48 (dd, 1H, J6',5' = 6.9 Hz, H-6'), 7.25–7.31 (m, 2H, H-3'/4'), 7.12–7.21 (m, 2H, H-3/5), 6.90 (d, 1H, J6,5 = 8.7 Hz, H-6), 5.17 (s, 2H, -OCH2), 2.23 (s, 3H, CH3); EI MS: m/z (%) 320 (M+, 12), 179 (80), 125 (100), 89 (75.8). Anal. Calcd for C16H14ClFN2O2, C = 59.91, H = 4.40, N = 8.73. Found: C = 59.89, H = 4.39, N = 8.72.

2-(4-Chloro-2-methylphenoxy)-N'-[3-thienylmethylidene]acetohydrazide (12). Yield: 68%; 1H-NMR (DMSO-d6) δ: 8.07 (s, 1H, -N=CH), 7.12–7.24 (m, 3H, H-3, 5, 6), 6.73–8.92 (m, 3H, H-2'/4'/5'), 5.10 (s, 2H, -OCH2), 2.22 (s, 3H, CH3); EI MS: m/z (%) 308 (M+, 15), 199 (10), 155 (45), 125 (100). Anal. Calcd for C14H13ClN2O2S, C = 54.46, H = 4.24, N = 9.07. Found: C = 54.45, H = 4.22, N = 9.04.

2-(4-Chloro-2-methylphenoxy)-N'-[-2-furylmethylidene]acetohydrazide (13). Yield: 70%; 1H-NMR (DMSO-d6) δ: 8.17 (s, 1H, -N=CH), 7.11–7.23 (m, 2H, H-3,5), 6.90 (d, 2H, J3',4' = J5',4' = 3.3 Hz, H-3'/5'), 6.81 (d, 1H, J6,5 = 8.7 Hz, H-6), 6.62 (dd, 1H, J4,3 = J4,5 = 3.3 Hz, 4'), 5.07 (s, 2H, -OCH2), 2.22 (s, 3H, -CH3); EI MS: m/z (%) 292 (M+, 35), 155 (50), 151 (80.), 125 (100). Anal. Calcd for C14H13ClN2O3, C = 57.44, H = 4.48, N = 9.57. Found: C = 57.44, H = 4.46, N = 9.56.

2-(4-Chloro-2-methylphenoxy)-N'-[-(5-methyl-2-furyl)methylidene]acetohydrazide (14). Yield: 72%; 1H-NMR (DMSO-d6) δ 8.07 (s, 1H, -N=CH), 7.11–7.23 (m, 2H, H-3,5), 6.88 (d, 1H, J5,6 = 8.7 Hz, 6), 6.62 (dd, 1H, J4,3 = J5,6 = 3.3 Hz, 4'), 5.07 (s, 2H, -OCH2), 2.22 (s, 3H, -CH3); EI MS: m/z (%) 306 (M +, 20), 65 (30), 137 (100), 125 (40). Anal. Calcd for C15H15ClN2O3, C = 58.73, H = 4.93, N = 9.13. Found: C = 58.70, H = 4.96, N = 9.10.

2-(4-Chloro-2-methylphenoxy)-N'-[-(2,3-dihydroxyphenyl)methylidene]acetohydrazide (15). Yield: 76%; 1H-NMR (DMSO-d6) δ 8.41 (s, 1H, -N=CH), 7.24 (m, 1H, Hz H-6), 7.13 (d, 1H, J3,5 = 2.7 Hz, H-3), 6.90 (d, 1H, J5,6 = 8.7 Hz, H-6'), 6.83 (d, 1H, J6,5 = 8.7 Hz, H-6), 6.71 (m, 2H, H-3'/4'), 5.15 (s, 2H, -OCH2), 2.19 (s, 3H, -CH3); EI MS: m/z (%) 334 (M+, 9), 177 (10), 151 (80.), 125 (100). Anal. Calcd for C16H15ClN2O4, C = 57.41, H = 4.5, N = 8.37. Found: C = 57.40, H = 3.50, N = 8.36.
(37.9). Anal. Calcd for C_{17}H_{16}ClN_{2}O_{3}, C = 61.36, H = 5.15, N = 8.42. Found: C = 61.34, H = 5.14, N = 8.40.

2-(4-Chloro-2-methylphenoxy)-N'-[(3-hydroxyphenyl)methylidene]acetohydrazide (18). Yield: 61%; \(^1\)H-NMR (DMSO-\(d_6\)) \(\delta\) 8.18 (s, 1H, -N=CH), 7.04–7.24 (m, 5H, Ar-H), 6.79–6.89 (m, 2H, H-5-6), 5.14 (s, 2H, -OCH\(_2\)), 2.22 (s, 3H, -CH\(_3\)); EI MS: \(m/z\) (%) 318 (M\(^+\), 97.6), 177 (100), 155 (71), 125 (65.3). Anal. Calcd for C\(_{16}\)H\(_{15}\)ClN\(_2\)O\(_3\), C = 60.29, H = 4.74, N = 8.79. Found: C = 69.27, H = 3.49, N = 8.34.

2-(4-Chloro-2-methylphenoxy)-N'-[(1-(2,4,6-trihydroxyphenyl)ethylidene]acetohydrazide (19). Yield: 62%; \(^1\)H-NMR (DMSO-\(d_6\)) \(\delta\): 8.16 (s, 1H, -N=CH), 7.21 (br.s, 2H, H-3'/5'), 7.16–7.23 (m, 2H, H-3/5), 6.86 (d, \(J_{6,5} = 8.7\) Hz, 1H, H-6), 4.98 (s, 2H, -OCH\(_2\)), 2.18 (s, 3H, CH\(_3\)), 1.93 (s, 3H, CH\(_3\)); EI MS: \(m/z\) (%) 254 (M\(^+\), 100), 155 (91.2), 142 (75.5), 125 (100), 113 (100), 99 (86.6).

2-(4-Chloro-2-methylphenoxy)-N'-[(4-hydroxyphenyl)methylidene]acetohydrazide (20). Yield: 72%; \(^1\)H-NMR (DMSO-\(d_6\)) \(\delta\): 7.88 (s, 1H, -N=CH), 7.52 (d, 2H, d, 2H, \(J_{2',3'} = J_{6',5'} = 8.7\) Hz, H-2'/6'), 7.24 (d, 1H, \(J_{5,6} = 8.1\) Hz, H-5), 7.13(d, 1H, \(J = 2.4\) Hz, H-3), 6.88 (d, 2H, \(J_{3,2'} = J_{5',6'} = 8.7\) Hz, H-3'/5'), 6.79 (d, 1H, \(J_{6,5} = 8.7\) Hz, H-6), 5.11 (s, 2H, -OCH\(_2\)), 2.22 (s, 3H, CH\(_3\)); EI MS: \(m/z\) (%) 318 (M\(^+\), 9.30), 199 (10.78), 177 (27.43), 155 (100), 125 (100), 77 (100). Anal. Calcd for C\(_{16}\)H\(_{15}\)ClN\(_2\)O\(_3\), C = 60.29, H = 4.74, N = 8.79. Found: C = 69.26, H = 3.50, N = 8.34.

2-(4-Chloro-2-methylphenoxy)-N'-[-1-naphthylmethylidene]acetohydrazide (21). Yield: 68%; 1H-NMR (DMSO-\(d_6\)) \(\delta\): 8.66 (s, 1H, N=CH), 8.83 (d, \(J = 8.1\) Hz 1H, H-8'), 8.61 (d, \(J_{4',3'} = 8.4\) Hz, H-4'), 8.02 (br, d, 2H, \(J = 7.8\) Hz, H-2'/5'), 7.93 (d, 1H, \(J_{3,4'} = 7.8\) Hz, H-3') 7.65 (m, 2H, H-2'/5'), 7.56 (t, 2H, \(J_{5',6'} = J_{8',7'} = 9.6\) Hz, H-5'/8'), 7.22 (s, 1H, H-1'), 7.17 (d, 1H, \(J_{7',8'} = 8.7\) Hz, H-7'), 6.92 (m, 2H, H-5/6), 5.23 (s, 2H, OCH\(_2\)), 2.23 (s, 3H, CH\(_3\)); EI MS: \(m/z\) (%) 352 (M\(^+\), 100), 211 (65), 199. Anal. Calcd for C\(_{20}\)H\(_{17}\)ClN\(_2\)O\(_2\), C = 68.09, H = 4.86, N = 7.94. Found: C = 68.06, H = 4.84, N = 7.92.

2-(4-Chloro-2-methylphenoxy)-N'-[-2-naphthylmethylidene]acetohydrazide (22). Yield: 70%; 1H-NMR (DMSO-\(d_6\)) \(\delta\): 8.43 (s, 1H, N=CH), 8.16 (t, 2H, \(J = 8.4\) Hz, H-3',6'), 7.93 (d, 1H, \(J_{3,4'} = 7.8\) Hz, H-3') 7.65 (m, 2H, H-6'/7'), 7.56 (t, 2H, \(J_{5',6'} = J_{8',7'} = 9.6\) Hz, H-5'/8'), 7.22 (s, 1H, H-1'), 7.17 (d, 1H, \(J_{7',8'} = 8.7\) Hz, H-7'), 6.92 (m, 2H, H-5/6), 5.23 (s, 2H, OCH\(_2\)), 2.23 (s, 3H, CH\(_3\)); EI MS: \(m/z\) (%) 352 (M\(^+\), 72), 211 (60) 199 (35), 169 (45), 153 (100), 127 (60). Anal. Calcd for C\(_{20}\)H\(_{17}\)ClN\(_2\)O\(_2\), C = 68.09, H = 4.86, N = 7.94. Found: C = 68.04, H = 4.85, N = 7.91.

2-(4-Chloro-2-methylphenoxy)-N'-[(5-Chloro-2-hydroxyphenyl)methylidene]acetohydrazide (23). Yield: 66%; \(^1\)H-NMR (DMSO-\(d_6\)) \(\delta\): 8.23 (s,1H, -N=CH), 7.70 (d, 1H, \(J_{6,4'} = 2.7\) Hz H-6'),7.12–7.23 (m, 2H, H-3/5), 7.31 (m, 1H, H-4'), 6.93 (d, 1H, \(J_{3,4'} = 7.8\) Hz, H-3'),6.86 (d, 1H, \(J_{6,5} = 8.7\) Hz, H-6), 5.17 (s, 2H, -OCH\(_2\)), 2.22 (s, 3H, -CH\(_3\)); EI MS: \(m/z\) (%) 352 (M\(^+\), 83.8), 210 (7.4), 197 (62.6), 155 (100), 141 (46.8). Anal. Calcd for C\(_{16}\)H\(_{15}\)Cl\(_2\)N\(_2\)O\(_3\), C = 54.41, H = 4.00, N = 7.93. Found: C = 54.39, H = 4.00, N = 7.92.

2-(4-Chloro-2-methylphenoxy)-N'-[1(2,4-dihydroxy-5-nitrophenyl)ethylidene]acetohydrazide (24). Yield: 70%; \(^1\)H-NMR (DMSO-\(d_6\)) \(\delta\): 8.23 (s, 1H, H-6'), 7.17–7.23 (m, 2H, H-3/5), 6.89 (d, 1H, \(J_{6,5} = 8.7\) Hz,
H-6), 6.51 (s, 1H, H-3'), 4.82 (s, 2H, -OCH₂), 2.26 (s, 3H, CH₃), 2.21 (s, 3H, CH₃); EI MS: m/z (%) 392 (M⁺, 64.4), 251 (72.1), 237 (100), 125 (89.9), 77 (68.5). Anal. Calcd for C₁₆H₁₄ClN₃O₆, C = 50.60, H = 3.72, N = 11.07. Found: C = 50.57, H = 3.70, N = 11.05.

2-(4-Chloro-2-methylphenoxy)-N’-[4-(4-hydroxy-3-methoxyphenyl)methylidene]acetohydrazide (25). Yield: 59%; 1H-NMR (DMSO-d₆) δ 8.11 (s, 1H, -N=CH), 7.12–7.23 (m, 2H, H-3/5), 7.03 (s, 1H, H-2’), 6.86–6.96 (m, 2H, H-5’/6’), 6.83 (d, 1H, J₆,₅ = 8.7 Hz, H-6), 5.12 (s, 2H, -OCH₂), 3.78 (s, 3H, OCH₃), 2.23 (s, 3H, CH₃); EI MS: m/z (%) 348 (M⁺, 88.5), 207 (51), 193 (100), 155 (43), 125 (46.9). Anal. Calcd for C₁₇H₁₇ClN₂O₄, C = 58.54, H = 4.91, N = 8.03. Found: C = 58.53, H = 4.90, N = 8.02.

4. Conclusions

A number of potential lead molecules 1, 5, 7, 8, 11, 12, 15, 21, and 22 have been identified as β-glucuronidase inhibitors and it is anticipated that by slight synthetic modification in these molecules, some new most active β-glucuronidase inhibitors can be developed.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/19/7/8788/s1.

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Author Contributions

All authors equally contributed.

Conflicts of Interest

The authors declare no conflict of interest.

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