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Research Article

Synthesis, characterization and pharmacokinetic studies of 4-(3-aryl-1, 6-dihydro-6-iminopyridazin-1-yl)butanoic acid hydrochlorides

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ABSTRACT

A series of six title compounds have been prepared using 3-amino-6-chloropyridazine as starting material collected from commercial sources and were characterized by IR, ^{1}H NMR, and high-resolution mass spectral (HRMS) data. The method involves three steps: Suzuki-Miyaura cross-coupling reaction, *N(2)*-alkylation, and acid hydrolysis, respectively, to obtain final products with good yields. According to Lipinski's rule of five and Veber's rule, pharmacokinetics studies of the synthesized compounds showed that all the parameters were in between the permissible limits. The toxicity parameters were low for the compounds to act as drugs.

Introduction

Heterocyclic compounds containing nitrogen are vital structural units of biologically active natural products and are important for medicinal compounds. The 3-aminopyridazine backbone has proved to have many synthetic and biological applications from a pharmaceutical perspective (Wermuth, 1998; Maes et al., 2000). γ-Aminobutyric acid (GABA) (Fig. 1) is the major inhibitory neurotransmitter in the central nervous system of animals (Bowery and Enna, 2000; Chebib and Johnston, 2000; Oslen and Sieghart, 2009). Compounds based on pyridazine moiety such as Gabazine (SR 95531) (Ueno et al., 1997) and Minaprine (Wermuth et al., 1987) (Fig. 1) act as competitive antagonists for mammalian GABA receptors (Zhu et al., 2018). The arylpyridazine moiety of the iminopyridazine analogs plays a vital role in exerting their GABA receptor antagonistic activity. Some synthesized aminopyridazine derivatives of GABA function as selective $GABA_A$ receptor antagonists for rat brain membrane (Wermuth et al., 1987). Arylaminopyridazine GABA derivatives exhibited antagonist properties in *Ascaris suum* GABA receptors (Duittoz and Martin, 1991; Martin et al., 1995). Gavande et al. (2010) reported a four-step synthetic procedure for gabazine in a condition of microwave irradiation starting with 3,6-dichoropyridazine. (Iqbal et al., 2011) reported several gabazine-based iminopyridazines as $GABA_A$ receptor antagonists. Rahman et al., recently reported iminopyridazine compounds, which block the function of the GABA receptor chloride channel and act as competitive antagonists for insect GABA receptors (Rahman et al., 2012; Rahman et al., 2014). A recent study showed that iminopyridazine butanoic acid derivatives act as competitive antagonists for housefly GABA receptors

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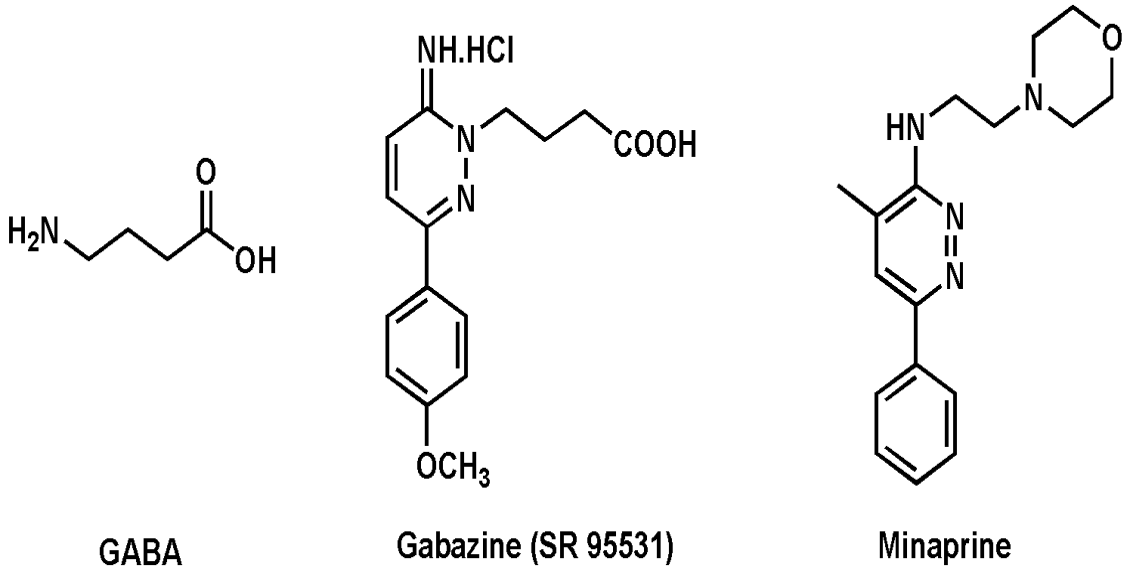
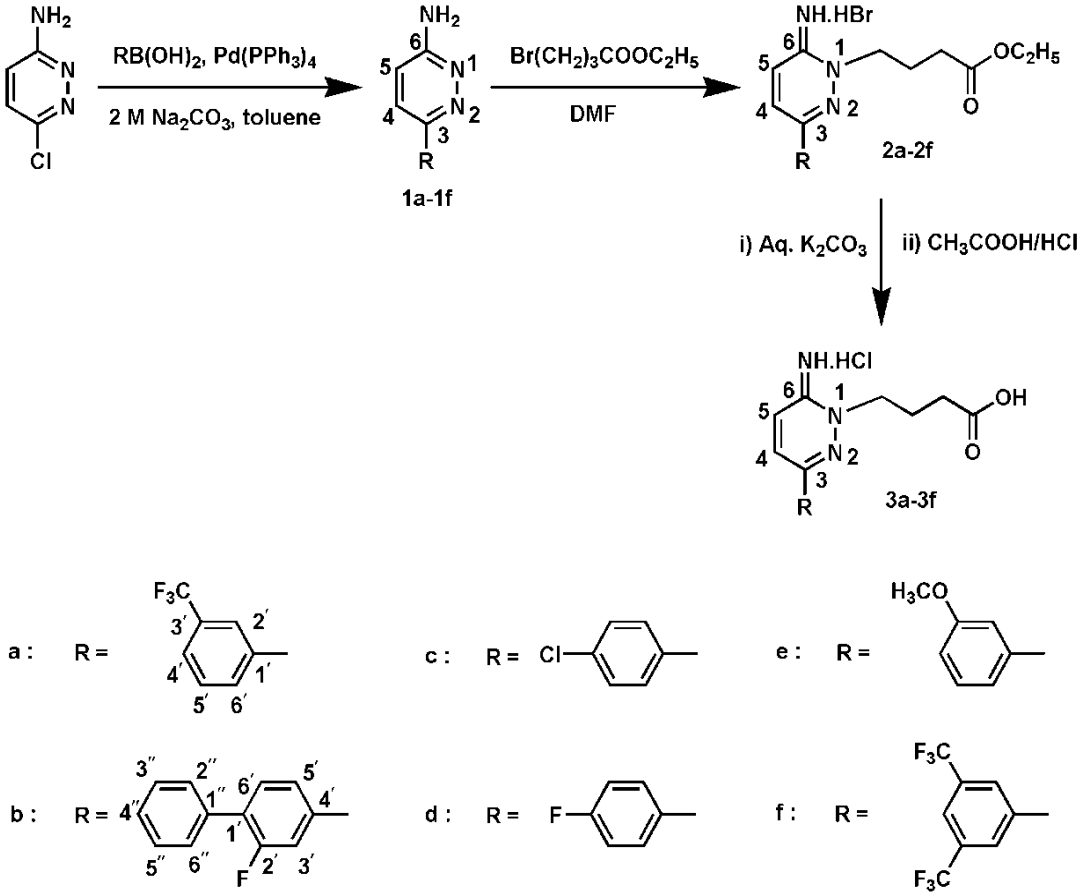


Fig. 1. Structures of inhibitory neurotransmitter GABA and two GABA receptor antagonists Gabazine (SR95531) and Minaprine, having pyridazine moiety.

(Rahman et al., 2021). Recently, we reported the synthesis of several iminopyridazine butyronitrle hydrobromides (Rahman et al., 2020). As gabazine based 3- substituted iminopyridazine of GABA functions as competitive antagonists for insect and mammalian GABA receptors, efforts continue to synthesize iminopyridazine carboxylic acid type compounds. In this study, six iminopyridazine butanoic acid hydrochloride derivatives were synthesized, and their structures were determined using different spectroscopic methods. Pharmacokinetic studies were performed using admetSAR. ADME (Absorption, Distribution, Metabolism, and Excretion) and toxicity properties of the synthesized compounds were determined to know the drug-likeness properties.

Experimental

An SMP10 apparatus was employed to determine the melting points of the synthesized compounds and remain uncorrected. Recording of the samples infrared (IR) spectra were done within the range of 4000-400 cm^{-1} by the SHIMADZU IR Tracer-100 infrared spectrophotometer and ran as KBr pellets. ^1H NMR spectra of the samples were recorded on a BRUKER 400 MHz NMR spectrometer. Deuterated chloroform ($CDCl_3$) and dimethyl sulfoxide (DMSO-d_6) were used as NMR solvents. Chemical shifts (δ values) are written in ppm relative to TMS (tetramethylsilane), and coupling constants (J values) are stated in Hz (Hertz). Expression of spin multiplicities were as follows: singlet (s), doublet (d), triplet (t), quartet (q), quintet (qn), and multiplet (m). The HRMS (high-resolution mass spectra) were recorded at Kumamoto University (analytical center), Kumamoto, Japan. Chemicals were obtained from TCI Chemical Industries, Ltd (India) and were used without any purification.



Scheme 1. Synthesis of 4-(3-aryl-1,6-dihydro-6-iminopyridazin-1-yl)butanoic acid hydrochlorides.

General procedure for the synthesis of 3-amino-6-arylpyridazines (1a-1f)

A mixture of 3-amino-6-chloropyridazine (259 mg, 2.0 mmole), arylboronic acid (2.2 mmole), tetrakis(triphenylphosphine)palladium (0) (70 mg) and 2 M aq. sodium carbonate (Na_2CO_3) solution (2.2 mL) in toluene (20 mL) was stirred at room temperature in an inert (nitrogen) condition. The mixture was refluxed with stirring under the inert condition before completing the reaction (Checked by TLC). The reaction mixture was cooled to room temperature and was evaporated using a rotary vacuum evaporator. EtOAc (60 mL) was added to the suspension and was put in an ultrasonic bath for 5 min. The suspension was filtered, and the filter paper was washed properly with 150 mL of EtOAc. The filtrate was dried using a rotary vacuum evaporator. Silica gel column chromatography was applied for purification to yield corresponding 3-amino-6-arylpyridazines **1a-1f**. We reported the synthesis of **1c**, **1d**, **1e**, and **1f** in a recent study (Rahman et al., 2020), and hence, data are not mentioned here.

3-Amino-6-(3-trifluromethylphenyl)pyridazine (1a). Yield (435.0 mg, 61%); R_f = 0.58 (EtOAc); mp 116-118 °C; IR (KBr): ν cm^{-1} 3440 (N-H stretch), 3120 (N-H stretch), 1600 ((N-H bend), ^{1}H NMR ($CDCl_3$): δ8.20 (1H, s, H-2′), 8.13 (1H, d, J = 7.6 Hz, H-4), 7.69 (1H, t, J = 5.2 Hz, H-5′), 7.65 (1H,

d, *J* = 8.8 Hz, H-5), 7.47-7.43 (2H, m, H-4′, H-6′), 6.87 (2H, s, NH_2). FAB HRMS (acetone/NBA) calcd. for $C_{11}H_9N_3F_3$ 240.0749 $[M+H]^+$. Found 240.0759.

3-Amino-6-(2-fluro-4-biphenylyl)pyridazine (1b). Yield (116.6 mg, 22%); R_f = 0.71 (EtOAc); mp 154-156 °C; IR (KBr): ν cm^{-1} 3440 (N-H stretch), 3120 (N-H stretch), 1600 (N-H bend); ^{1}H NMR (DMSO-d_6): δ 7.92-7.88 (2H, m, H-3′, H-5′), 7.64-7.60 (4H, m, H-2′′, H-3′′, H-5′′, H-6′′), 7.52-7.49 (2H, m, H-4, H-6′), 7.42 (1H, t, *J* = 7.6 Hz, H-4′′), 6.89 (1H, d, *J* = 8.0 Hz, H-5), 6.58 (2H, s, NH_2). FAB HRMS (acetone/NBA) calcd. for $C_{16}H_{13}N_3F$ 266.1094 $[M+H]^+$. Found 266.1090.

General procedure for the synthesis of ethyl 4-(3-aryl-1,6-dihydro-6-iminopyridazin-1-yl) butanoate hydrobromides (2a-2f)

A mixture of 3-amino-6-arylpyridazine (1 mmole), ethyl 4-bromobutanoate (234 mg, 1.2 mmole), and *N*,*N*-dimethylformamide (1.0 mL) was heated at 80 ^{0}C for 30 h. After cooling, the collected precipitate was recrystallized from methanol (MeOH) and ethyl acetate (EtOAc) to yield **2a-2f**.

Ethyl 4-[1,6-dihydro-6-imino-3-(3-triflurome-thylphenyl)pyridazin-1-yl]butanoate hydro-bromide (2a). Yield (394.9 mg, 91%); R_f = 0.65 (MeOH); mp 217-219 °C; IR (KBr): ν cm^{-1} 3440 (NH), 1760 (C=O), 1650 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.20 (2H, bs, imino H), 8.50 (1H, d, *J* = 9.2 Hz, H-4), 8.25 (2H, d, *J* = 7.6 Hz, H-4′, H-6′), 7.90 (1H, d, *J* = 7.6 Hz, H-2′), 7.79 (1H, t, *J* = 7.8 Hz, H-5′), 7.70 (1H, d, *J* = 9.6 Hz, H-5), 4.37 (2H, t, *J* = 6.8 Hz, $COCH_2CH_2CH_2$), 3.90 (2H, q, *J* = 7.2 Hz, OCH_2CH_3), 2.48 (2H, t, *J* = 7.2 Hz, $COCH_2CH_2CH_2$), 2.06 (2H, qn, *J* = 6.8 Hz, $COCH_2CH_2CH_2$), 1.03 (3H, t, *J* = 7.0 Hz, OCH_2CH_3). FAB HRMS (acetone/NBA) calcd. for $C_{17}H_{19}F_3N_3O_2$ 354.1429 $[M-Br]^+$. Found 354.1441.

Ethyl 4-[3-(2-fluro-4-biphenylyl)-1,6-dihydro-6-iminopyridazin-1-yl]butanoate hydrobromide (2b). Yield (101.2 mg, 22%); R_f = 0.45 (MeOH); mp 240-244 °C; IR (KBr): ν cm^{-1} 3440 (NH), 1720 (C=O), 1660 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.20 (2H, bs, imino H), 8.49 (1H, d, *J* = 9.6 Hz, H-4), 7.94 (2H, t, *J* = 8.4 Hz, H-2′′, H-6′′), 7.76 (1H, t, *J* = 8.0 Hz, H-4′′), 7.68 (1H, d, *J* = 9.6 Hz, H-5), 7.62 (2H, d, *J* = 7.6 Hz, H-3′′, H-5′′), 7.53 (2H, t, *J* = 7.6 Hz, H-3′, H-5′), 7.47 (1H, d, *J* = 7.6 Hz, H-6′), 4.39 (2H, t, *J* = 6.8 Hz, $COCH_2CH_2CH_2$), 3.97 (2H, q, *J* = 7.0 Hz, OCH_2CH_3), 2.51 (2H, t, *J* = 4.3 Hz, $COCH_2CH_2CH_2$), 2.12 (2H, qn, *J* = 6.8 Hz, $COCH_2CH_2CH_2$), 1.10 (3H, t, *J* = 7.2 Hz, OCH_2CH_3). FAB HRMS (acetone/NBA) calcd. for $C_{22}H_{23}FN_3O_2$ 380.1774 $[M-Br]^+$. Found 380.1784.

Ethyl 4-[3-(4-chlorophenyl)-1,6-dihydro-6-imino-pyridazin-1-yl]butanoate hydrobromide (2c). Yield (240.3 mg, 60%); R_f = 0.60 (MeOH); mp 246-248 °C; IR (KBr): ν cm^{-1} 3440 (NH), 1720 (C=O), 1650 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.20 (2H, bs, imino H), 8.42 (1H, d, *J* = 9.6 Hz, H-4), 8.00 (2H, d, *J* = 7.6 Hz, H-3′, H-5′), 7.67 (3H, t, *J* = 6.8 Hz, H-5, H-2′, H-6′), 4.37 (2H, t, *J* = 6.8 Hz, $COCH_2CH_2CH_2$), 3.95 (2H, q, *J* = 6.8 Hz, OCH_2CH_3), 2.51 (2H, t, *J* = 6.8 Hz, $COCH_2CH_2CH_2$), 2.10 (2H, qn, *J* = 6.8 Hz, $COCH_2CH_2CH_2$), 1.08 (3H, t, *J* = 6.8 Hz, OCH_2CH_3). FAB HRMS (acetone/NBA) calcd. for $C_{16}H_{19}ClN_3O_2$ 320.1166 $[M-Br]^+$. Found 320.1173.

Ethyl 4-[1,6-dihydro-3-(4-flurophenyl)-6-imino-pyridazin-1-yl]butanoate hydrobromide (2d). Yield (188.2 mg, 49%); R_f = 0.65 (MeOH); mp 117-119 °C; IR (KBr): ν cm^{-1} 3440 (N-H), 1720 (C=O), 1630 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.00 (2H, bs, imino H), 8.42 (1H, d, J = 9.6 Hz, H-4), 8.04 (2H, dd, J = 5.4, 3.6 Hz, H-3′, H-5′), 7.68 (1H, d, J = 9.6 Hz, H-5), 7.45-7.41 (2H, m, H-2′, H-6′), 4.37 (2H, t, J = 6.8 Hz, $COC\underline{H}_2CH_2CH_2$), 3.95 (2H, q, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 2.50 (2H, t, J = 2.0 Hz, $COCH_2CH_2C\underline{H}_2$), 2.09 (2H, qn, J = 7.0 Hz, $COCH_2C\underline{H}_2CH_2$), 1.08 (3H, t, J = 7.0 Hz, $OCH_2C\underline{H}_3$). FAB HRMS (acetone/NBA) calcd. for $C_{16}H_{19}FN_3O_2$ 304.1461 $[M\text{-}Br]^+$. Found 304.1484.

Ethy l4-[1,6-dihydro-6-imino-3-(3-methoxy-phenyl)pyridazin-1-yl]butanoate hydrobromide (2e). Yield (257.4 mg, 65%); R_f = 0.53 (MeOH); mp 214-215 °C; IR (KBr): ν cm^{-1} 3400 (NH), 1700 (C=O), 1680 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.10 (2H, bs, imino H), 8.43 (1H, d, J = 9.6 Hz, H-4), 7.63 (1H, d J = 9.6 Hz, H-5), 7.55-7.49 (3H, m, H-2′, H-4′, H-5′), 7.15 (1H, d, J = 8.0 Hz, H-6′), 4.36 (2H, t, J = 7.1 Hz, $COC\underline{H}_2CH_2CH_2$), 3.95 (2H, q, J = 6.8 Hz, $OC\underline{H}_2CH_3$), 3.84 (3H, s, OCH_3), 2.54 (2H, t, J = 7.5 Hz, $COCH_2CH_2C\underline{H}_2$), 2.10 (2H, qn, J = 6.8 Hz, $COCH_2C\underline{H}_2CH_2$), 1.09 (3H, t, J = 7.2 Hz, $OCH_2C\underline{H}_3$). FAB HRMS (acetone/NBA) calcd. for $C_{17}H_{22}N_3O_3$ 316.1661 $[M\text{-}Br]^+$. Found 316.1669.

Ethyl 4-[3-(3,5-bistrifluromethylphenyl)-1,6-dihydro-6-iminopyridazin-1-yl]butanoate hydrobromide (2f). Yield (276.1 mg, 55%); R_f = 0.28 (MeOH); mp 190-192 °C; IR (KBr): ν cm^{-1} 3440 (NH), 1700 (C=O), 1660 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.40 (2H, bs, imino H), 8.81 (1H, d, J = 9.2 Hz, H-4), 8.76 (2H, d, J = 9.2 Hz, H-2′, H-6′), 8.51 (1H, s, H-4′), 7.89 (1H, d, J = 9.2 Hz, H-5), 4.59 (2H, t, J = 6.4 Hz, $COC\underline{H}_2CH_2CH_2$), 4.11 (2H, q, J = 7.2 Hz, $OC\underline{H}_2CH_3$), 2.69-2.65 (2H, m, $COCH_2CH_2C\underline{H}_2$), 2.26 (2H, qn, J = 6.8 Hz, $COCH_2C\underline{H}_2CH_2$), 1.23 (3H, t, J = 6.8 Hz, $OCH_2C\underline{H}_3$). FAB HRMS (acetone/NBA) calcd. for $C_{18}H_{18}N_3F_6O_2$ 422.1303 $[M\text{-}Br]^+$. Found 422.1312.

General procedure for the synthesis of 4-(3-aryl-1,6-dihydro-6-iminopyridazin-1-yl) butanoic acid hydrochlorides (3a-3f)

Ethyl 4-(3-aryl-1,6-dihydro-6-iminopyridazin-yl) butanoate hydrobromide (100 mg) was dissolved in aq. K_2CO_3 to make the solution alkaline. The solution was then extracted with EtOAc. The organic layer was dried with anhydrous Na_2SO_4 and was concentrated in a rotary vacuum evaporator to give free base esters, ethyl 4-(3-aryl-1,6-dihydro-6-iminopyridazin-1-yl)butanoates. Concentrated hydrochloric acid (3 mL) in glacial acetic acid (10 mL) was added to the free base ester and heated at 100 ^{0}C for approximately 60 h. After cooling, the reaction mixture was evaporated in a rotary vacuum evaporator to dry. The residue was recrystallized with MeOH and EtOAc to yield 4-(3-aryl-1,6-dihydro-6-iminopyridazin-1-yl)butanoic acid hydrochlorides **3a-3f**.

4-[1,6-Dihydro-6-imino-3-(3-trifluromethyl-pyridazin-1-yl]butanoic acid hydrochloride (3a). Yield (49.0 mg, 49%); R_f = 0.56 (MeOH); mp 257-260 °C; IR (KBr): ν cm^{-1} 3280 (NH), 3040-2850 (OH), 1740 (C=O), 1680 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.20 (2H, bs, imino H), 8.46 (1H, d, J = 9.6 Hz, H-4), 8.21 (2H, s, H-2′, H-6′),

7.86 (1H, d, *J* = 7.6 Hz, H-5), 7.80-7.73 (2H, m, H-4′, H-5′), 4.37 (2H, t, *J* = 7.0 Hz, $COC\underline{H}_2CH_2CH_2$), 2.38 (2H, t, *J* = 7.0 Hz, $COCH_2CH_2C\underline{H}_2$), 2.01 (2H, qn, *J* = 7.0 Hz, $COCH_2C\underline{H}_2CH_2$). FAB HRMS (Acetone/NBA) calcd. for $C_{15}H_{15}F_3N_3O_2$ 326.1116 $[M\text{-}Cl]^+$. Found 326.1119.

4-[1,6-Dihydro-3-(2-fluro-4-biphenylyl)-6-iminopyridazin-1-yl]butanoic acid hydrochloride (3b). Yield (53.0 mg, 53%); R_f = 0.62 (MeOH), mp 240-244 °C; IR (KBr): ν cm^{-1} 3400 (NH), 3080-2920 (OH), 1720 (C=O), 1660 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.20 (2H, bs, imino H), 8.49 (1H, d, *J* = 9.6 Hz, H-4), 7.95 (2H, t, *J* = 7.2 Hz, H-2″, H-6″), 7.76 (2H, d, *J* = 9.2 Hz, H-5, H-3′), 7.62 (2H, d, *J* = 7.6 Hz, H-5′, H-6′), 7.53 (2H, dd, *J* = 7.6, 7.2 Hz, H-3″, H-5″), 7.47 (1H, d, *J* = 7.2 Hz, H-4″), 4.40 (2H, t, *J* = 6.8 Hz, $COC\underline{H}_2CH_2CH_2$), 2.45 (2H, t, *J* = 6.8 Hz, $COCH_2CH_2C\underline{H}_2$), 2.09 (2H, qn, *J* = 7.2 Hz, $COCH_2C\underline{H}_2CH_2$). FAB HRMS (Acetone/NBA) calcd. for $C_{20}H_{19}FN_3O_2$ 352.1461 $[M\text{-}Cl]^+$. Found 352.1462.

4-[3-(4-Chlorophenyl)-1,6-dihydro-6-iminopyridazin-1-yl]butanoic acid hydrochloride (3c). Yield (55.0 mg, 55%); R_f = 0.47 (MeOH); mp 221-223 °C; IR (KBr): ν cm^{-1} 3440 (NH), 2950-2830 (OH), 1700 (C=O), 1650 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.20 (2H, bs, imino H), 8.41 (1H, d, *J* = 9.6 Hz, H-4), 8.00 (2H, d, *J* = 7.6 Hz, H-3′, H-5′), 7.72 (1H, d, *J* = 9.2 Hz, H-5), 7.65 (2H, d, *J* = 7.6 Hz, H-2′, H-6′), 4.37 (2H, t, *J* = 6.8 Hz, $COC\underline{H}_2CH_2CH_2$), 2.43 (2H, t, *J* = 7.2 Hz, $COCH_2CH_2C\underline{H}_2$), 2.06 (2H, qn, *J* = 6.8 Hz, $COCH_2C\underline{H}_2CH_2$). FAB HRMS (acetone/NBA) calcd. for $C_{14}H_{15}ClN_3O_2$ 292.0853 $[M\text{-}Cl]^+$. Found 292.0862.

4-[1,6-Dihydro-3-(4-flurophenyl)-6-iminopyridazin-1-yl]butanoic acid hydrochloride (3d). Yield (40.0 mg, 40%); R_f = 0.56 (MeOH); mp 245-247 °C; IR (KBr): ν cm^{-1} 3280 (NH), 3080-2840 (OH), 1720 (C=O), 1620 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.20 (2H, bs, imino H), 8.38 (1H, d, *J* = 9.6 Hz, H-4), 8.02 (2H, dd, *J* = 6.0, 2.0 Hz, H-3′, H-5′), 7.72 (1H, d, *J* = 9.2 Hz, H-5), 7.39 (2H, t, *J* = 8.4 Hz, H-2′, H-6′), 4.35 (2H, t, *J* = 6.8 Hz, $COC\underline{H}_2CH_2CH_2$), 2.41 (2H, t, *J* = 7.2 Hz, $COCH_2CH_2C\underline{H}_2$), 2.04 (2H, qn, *J* = 7.2 Hz, $COCH_2C\underline{H}_2CH_2$). FAB HRMS (Acetone/NBA) calcd. for $C_{14}H_{15}FN_3O_2$ 276.1148 $[M\text{-}Cl]^+$. Found 276.1152.

4-[1,6-Dihydro-6-imino-3-(3-methoxyphenyl) pyridazin-1-yl]butanoic acid hydrochloride (3e). Yield (52.0 mg, 52%); R_f = 0.67 (MeOH); 198-200 °C; IR (KBr): ν cm^{-1} 3190 (NH), 3070-2820 (OH), 1730 (C=O), 1650 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.40 (2H, bs, imino H), 8.41 (1H, d, *J* = 9.6 Hz, H-4), 7.66 (1H, t, *J* = 9.2 Hz, H-2′), 7.55 (1H, d, *J* = 7.6 Hz, H-5), 7.48 (1H, t, *J* = 6.4 Hz, H-5′), 7.34 (1H, dd, *J* = 7.6, 6.4 Hz, H-6′), 6.96 (1H, d, *J* = 7.6 Hz, H-4′). 4.36 (2H, t, *J* = 7.2 Hz, $COC\underline{H}_2CH_2CH_2$), 3.83 (3H, s, OCH_3), 2.43 (2H, t, *J* = 7.2 Hz, $COCH_2CH_2C\underline{H}_2$), 2.07 (2H, qn, *J* = 7.2 Hz, $COCH_2C\underline{H}_2CH_2$). FAB HRMS (acetone/NBA) calcd. for $C_{15}H_{18}N_3O_3$ 288.1348 $[M\text{-}Cl]^+$. Found 288.1366.

4-[3-(3,5-Bistrifluromethylphenyl)-1,6-dihydro-6-iminopyridazin-1-yl]butanoic acid hydrochloride (3f). Yield (70.0 mg, 70%); R_f = 0.61 (MeOH); mp 203-205 °C; IR (KBr): ν cm^{-1} 3280 (NH), 3160-3040 (OH), 1700 (C=O), 1680 (C=N); ^{1}H NMR (DMSO-d_6): δ 9.30 (2H, bs, imino H), 8.60 (2H, d, *J* = 6.4 Hz, H-2′, H-6′), , 8.53 (1H, d, *J* = 9.6 Hz, H-4), 8.33 (1H, s, H-4′), 7.86 (1H, d, *J* = 9.6 Hz,

H-5), 4.46 (2H, t, *J* = 6.4 Hz, $COCH_2CH_2CH_2$), 2.45 (2H, t, *J* = 7.2 Hz, $COCH_2CH_2CH_2$), 2.07 (2H, qn, *J* = 6.8 Hz, $COCH_2CH_2CH_2$). FAB HRMS (acetone/NBA) calcd. for $C_{16}H_{14}N_3O_2F_6$ 394.0990 $[M\text{-}Cl]^+$. Found 394.0979.

Pharmacokinetic, Toxicity, and Drug-likeness Properties

ADME (absorption, distribution, metabolism, and excretion) and solubility of synthesized compounds were assessed online using admetSAR, and the percentage of absorption (%ABS) was obtained from the following formula: %ABS = 109-(0.3459×TPSA) where TPSA is topological polar surface area. According to a previous report, Osiris Property Explorer was used to predict the overall toxicities (mutagenic, tumorigenic, irritant, and reproductive), drug-likeness, and drug-score of the compounds (Park et al., 2011).

Results and Discussion

A series of six 4-(3-aryl-1,6-dihydro-6-imino-pyridazin-1-yl)butanoic acid derivatives were synthesized by modifying the 3-position of pyridazine ring of gabazine with various aromatic substituents **3a-3f** (**Scheme 1**). γ-Aminobutyric acid (GABA) (**Fig. 1**) functions as an agonist for GABA receptors. The Ariëns theory for accessory binding sites proposed that agonists are often converted to antagonists if the hydrophobic moieties are included in the polar agonists (Wermuth et al., 1987). Moreover, these iminopyridazine butanoic acids act as a competitive antagonist for mammalian and insect GABA receptors. That scientific evidence directed us to synthesize more iminopyridazine butanoic acids by changing the 3-position of pyridazine ring of gabazine by various aromatic groups. Wermuth and co-workers reported the synthesis of **3c** and **3d** by a different process. In that procedure, hydrazine followed by the reduction with Raney nickel or NH_4OH-NH_4Cl was used to prepare 3-amino-6-arylpyridazine starting from 3-chloro-6-arylpyridazine (Wermuth et al., 1987). The process described in this study involves three steps taking 3-amino-6-chloropyridazine as starting material. Intermediates **1a-1f** were synthesized in 22-65% yields using the Suzuki-Miyaura cross-coupling reaction in the presence of Pd (0) catalyst according to previous reports (Maes et al., 2000; Guery et al., 2001; Maes et al., 2002; Rahman et al., 2020) (**Scheme 1**). We recently reported the synthesis of **1c**, **1d**, **1e**, and **1f** (Rahman et al., 2020). The maximum yield (65%) was obtained for 3,5-bis (trifluoromethyl) phenyl analog **1f** (data are not shown). The electron withdrawing nature of the substituent might favor the reaction. A relatively high yield (61%) was also obtained for compound **1a** due to the electron-withdrawing capacity of the trifluoromethylphenyl group. The same cross-coupling reaction did not proceed using 2-trifluromethyphenyl analog due to steric hindrance. In the IR spectrum of **1a**, two absorption bands appeared at 3440 and 3120 cm^{-1} for N-H stretching. The band at 1600 cm^{-1} was observed for N-H bending. In the ^1H NMR spectrum of **1a**, a one proton singlet at 8.20 ppm (1H, s, H-2′), a one proton doublet at 8.13 ppm (1H, d, *J* = 7.6 Hz, H-4), a one proton triplet at 7.69 ppm (1H, t, *J* = 5.2 Hz, H-5′), a one proton doublet at 7.65 ppm (1H, d, *J* = 8.8 Hz, H-5), and a two protons multiplet at 7.47-7.43 ppm (2H, m, H-4′, H-6′) appeared for aromatic protons. As H-4 was adjacent to the phenyl group, it was more

deshielded than H-5. The $-NH_2$ protons have appeared at 6.87 ppm as a singlet. The *N(2)*-alkylated compounds **2a-2f** were obtained in 22-91% yields by the reaction of **1a-1f** with ethyl 4-bromobutanoate. In the IR spectrum of **2a**, the NH, C=O, and C=N functional groups showed absorption bands at 3440, 1760, and 1650 cm^{-1}, respectively. For compound **2a**, a broad singlet at 9.20 ppm was found for imino protons (C=NH.H) in the ^1H NMR spectrum. Peaks at 8.50 ppm (1H, d, *J* = 9.2 Hz, H-4), 8.25 ppm (2H, d, *J* = 7.6 Hz, H-4′, H-6′), 7.90 ppm (1H, d, *J* = 7.6 Hz, H-2′), 7.79 ppm (1H, t, *J* = 7.8 Hz, H-5′), and 7.70 ppm (1H, d, *J* = 9.6 Hz, H-5) were found for aromatic protons. The signals for $OC\underline{H}_2CH_3$ and $OCH_2C\underline{H}_3$ protons were found at 3.90 ppm (2H, q, *J* = 7.2 Hz) and 1.03 ppm (3H, t, *J* = 7.0 Hz), respectively. The $>CH_2$ protons of the alkyl part were observed at 4.37 ppm (2H, t, *J* = 6.8 Hz, $COC\underline{H}_2CH_2CH_2$), 2.48 ppm (2H, t, *J* = 7.2 Hz, $COCH_2CH_2C\underline{H}_2$), and 2.06 ppm (2H, qn, *J* = 6.8 Hz, $COCH_2C\underline{H}_2CH_2$). Free base esters of the alkylated compounds were prepared using an aqueous K_2CO_3 solution. The hydrolysis of free base esters was carried out in acetic acid containing concentrated HCl at 100 ^oC to give final compounds 4-(3-aryl-1,6-dihydro-6-iminopyridazin-1-yl)butanoic acid hydrochlorides **3a-3f** in 40-70% yields. In the IR spectrum, the absorption bands for **3a** that appeared at 3280, 2880, 1740, and 1680 cm^{-1} were assigned to NH, OH, C=O, and C=N functional groups, respectively. The ^1H NMR spectrum for the analog **3a** showed a broad singlet at 9.20 ppm for imino protons. The aromatic protons appeared at 8.46 ppm (1H, d, *J* = 9.6 Hz, H-4), 8.21 ppm (2H, s, H-2′, H-6′), 7.86 ppm (1H, d, *J* = 7.6 Hz, H-5), and 7.80-7.73 ppm (2H, m, H-4′, H-5′). Protons for $>CH_2$ were found at 4.37 ppm (2H, t, *J* = 7.0 Hz, $COC\underline{H}_2CH_2CH_2$), 2.38 ppm (2H, t, *J* = 7.2 Hz, $COCH_2CH_2C\underline{H}_2$), and 2.01 ppm (2H, qn, *J* = 7.0 Hz, $COCH_2C\underline{H}_2CH_2$). The $>CH_2$ protons adjacent to the carboxylic group appeared downfield relative to other alkyl protons. High-resolution mass spectrometry (HRMS) is very selective in measuring the exact mass of a compound. The first step, intermediates **1**, contains an odd number of nitrogen atoms, but they displayed an even mass number in HRMS due to the addition of one hydrogen with molecular ion peaks. Similarly, the compounds **2a-2f** and **3a-3f** showed even masses, although they contain an odd number of nitrogen atoms as the molecular ion peaks appeared after eliminating Br and Cl, respectively. The calculated exact mass values of the synthesized compounds were well agreed with the mass found in HRMS, which confirmed the structures of the synthesized analogs.

Pharmacokinetic and Drug-likeness Properties

Many new drugs fail due to unfavorable ADME (Absorption, Distribution, Metabolism, and Excretion) and toxicity properties in clinical trials. Therefore, it is crucial to know pharmacokinetics and toxicity parameters for designing and developing new drugs. Lipinski's rule of five and Veber's rule are generally used to determine the drug-likeness properties of a compound, which include the parameters like molecular weight (MW), number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD), number of rotatable bonds (NROTB), lipophilicity (clog P), topological polar surface area (TPSA), solubility parameter (log S), and percentage of absorption (%ABS). ADMEprediction of the synthesized compounds was calculated using

admetSAR, and the obtained results are summarized in Table 1. It was found that all the parameters were in a range of acceptable limits without any violations of Lipinski's rule of five and Veber's rule. Toxicity risks profile, drug-likeness, and drug-score obtained from the analysis are summarized in Table 2. *In silico* toxicity calculation of all the synthesized compounds showed low toxicity risk. Drug-likeness characteristics of a compound are partly based on topological descriptors, fingerprints of molecular drug-likeness with other properties such as clog P, and molecular weights (Tetko, 2005). Drug score is one of the significant parameters combined with drug-likeness, clog P, log S, molecular weight, and toxicity risks to evaluate a compound's overall potential for qualifying as a drug. *In silico*, ADME, toxicity, drug-likeness, and drug-score (Table 2) of iminopyridazine butanoic acid analogs suggest that they would be pharma-cologically active compounds for the future development of a safe and effective drug.

Table 1. ADME predictions using Lipinski's rule of five, Veber's rule, solubility, and absorption parameters of the iminopyridazine butanoic acid analogs (3a-3f). Acceptable limits are given in parentheses.

Comp.	Lipinski's Violations	Lipinski's rule				Veber's rule			
		MW (≤ 500)	HBA (≤ 10)	HBD (≤ 5)	clogP (≤5)	NROTB (≤ 10)	TPSA (140 Å^2)	logS	%ABS
3a	0	361.75	4	2	3.33	5	76.75	-3.99	82.52
3b	0	387.84	4	2	4.12	6	78.98	-4.81	81.75
3c	0	328.20	4	2	2.97	5	76.75	-3.74	82.52
3d	0	311.74	4	2	2.46	5	76.75	-3.31	82.52
3e	0	323.78	5	2	2.32	6	85.98	-3.22	79.33
3f	0	429.75	4	2	4.35	5	76.75	-4.85	82.52

MW = molecular weight; HBA = number of hydrogen bond acceptors; HBD = number of hydrogen bond donors; NROTB = number of rotatable bonds; clogP = lipophilicity; TPSA = topological polar surface area; logS = solubility parameter; %ABS = percentage of absorption.

Table 2. *In silico* toxicity risks, drug-likeness, and drug-score of iminopyridazine butanoic acid analogs.

Comp.	Toxicity effects				Drug-likeness	Drug-score
	M	T	I	R		
3a	Low	Low	Low	Low	-4.4	0.45
3b	Low	Low	Low	Low	1.45	0.67
3c	Low	Low	Low	Low	4.19	0.90
3d	Low	Low	Low	Low	2.48	0.89
3e	Low	Low	Low	Low	2.55	0.90
3f	Low	Low	Low	Low	-19.5	0.38

M = mutagenic; T = tumorigenic; I = irritant; R = reproductive

Conclusion

The synthesis of 4-(3-aryl-1,6-dihydro-6-imino-pyridazin-1-yl)butanoic acid hydrochlorides were performed starting from 3-amino-6-chloropyridazine in three steps. As the γ-aminobutyric acid (GABA) derivatives types of compounds act as competitive antagonists for mammalian and insect GABA receptors, and pharmacokinetic data presented in this study would be proved helpful for discovering the future drug.

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