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Antimycobacterial Activity of Pyrimido[4,5-*b*]diazepine Derivatives

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Three series of novel 8,9-dihydro-7*H*-pyrimido[5,4-*b*][1,4]diazepines, **4a–d**, **5a–d**, and **7a–d**, were efficiently obtained in good yields using simple reaction methodologies. These pyrimidodiazepines were evaluated against 15 *Mycobacterium* spp. strains. Moderate activity in the inhibition of 13 microorganisms was obtained for the four compounds **4a**, **5a**, **5c**, and **5d**.

Keywords: 4,5-Diaminopyrimidines / Methylenedioxychalcones / Pyrimido[4,5-*b*]diazepine / Tuberculosis

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Introduction

Human tuberculosis (TB) is a contagious–infectious disease mainly caused by *Mycobacterium tuberculosis*, which is an aerobic pathogenic bacterium that establishes its infection usually in the lungs [1]. About one-third of the world's population is currently infected with *M. tuberculosis*; 10% of those infected will develop clinical disease, particularly those who are also infected with the human immunodeficiency virus (HIV) [1]. Although medical regimens exist for treating TB, they are far from ideal [2]. In addition, new drugs are needed to combat the increasing number of multidrug-resistant strains (MDR-TB) and extensively drug-resistant (XDR-TB) strains and HIV epidemics, leading to an increased need to understand the molecular mechanisms of drug action and drug resistance, which should provide significant insights into the development of new compounds [2]. In this effort, diazepine derivatives are scaffolds that have shown a broad spectrum of biological activities as anticancer, antibacterial, psychotropic, anticonvulsant, antiviral, and herbicidal agents; these facts present them as a promising source of new compound leaders [3]. Benzodiazepines are an important class of psychotherapeutic compounds. Although a benzene ring is usually needed for pharmacological activity, in recent years, some examples of a heterocyclic ring fused to the seven-membered diazepine ring system have appeared in the literature [4].

In previous papers [5], we reported on the synthesis of pyrimidodiazepines from the reaction of 4,5-diaminopyrimidines with chalcones. Because of the biological importance of these heterocycles, the aim of this study was the synthesis of the new pyrimidodiazepines **4**, **5**, and **7** by the reaction of 4,5-diaminopyrimidines **1** and **6** with chalcones **2** and **3** containing the dioxymethylene moiety, and the determination of their activity against 15 *Mycobacterium* spp., in comparison between strains and clinical isolates.

Results and discussion

Chemistry

The synthesis of pyrimidodiazepines **4**, **5**, and **7** was achieved by two different methodologies (Scheme 1): Compounds **4** and **5** were obtained by a mixture of the dihydrochloride of 2,4,5,6-tetraaminopyrimidine **1** (0.100 g, 0.47 mmol) and methylenedioxychalcone **2** or **3** (0.94 mmol) in methanol (30 mL) under reflux for 30 min. Compound **7** was obtained using microwave irradiation (120°C, 75 W; CEM Discovery) during 4–14 min, in the reaction of 2-methylthio-4,5,6-triaminopyrimidine **6** (0.60 mmol) and chalcone **3** (0.30 mmol) in dimethylformamide (DMF) (0.5 mL) and a catalytic amount of $BF_3{\cdot}OEt_2$. The structures of all new pyrimidodiazepines were appropriately established by the usual spectroscopic methods (see Experimental section).

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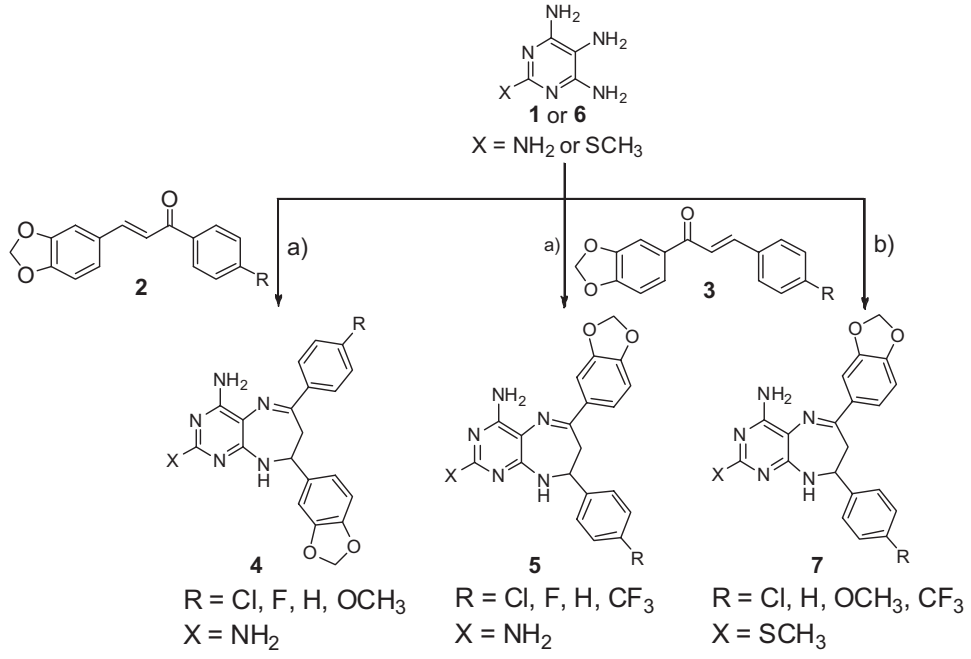

a) MeOH reflux, 30 minutes. **b)** $BF_3.OEt_2$ (cat.), DMF (5 mL), MW close vessel, 120ºC, 75 W, 4–14 minutes.

Compd	4a	4b	4c	4d	5a	5b	5c	5d	7a	7b	7c	7d
X	NH_2	NH_2	NH_2	NH_2	NH_2	NH_2	NH_2	NH_2	SCH_3	SCH_3	SCH_3	SCH_3
R	Cl	F	H	OCH_3	Cl	F	H	CF_3	Cl	H	OCH_3	CF_3

Scheme 1. Synthesis of pyrimidodiazepines **4a–d**, **5a–d**, and **7a–d**.

In the ^{1}H NMR spectra of the compounds, one proton on the diastereotopic center C-7 of the 1,4-diazepine ring appears, for compounds **4a–d**, as a doublet of doublets at $\delta = 3.07–3.13$ ppm, with a coupling constant of $J_{gem} = 14.4–14.8$ Hz, $J_{trans} = 7.2–7.6$ Hz, and the other diastereotopic proton in C-7 appears as a doublet at $\delta = 3.29–3.38$ ppm with a coupling constant of $J_{gem} = 14.4–14.8$ Hz. For compounds **5** and **7**, one proton on the diastereotopic center C-7 appears as a doublet at $\delta = 2.76–3.21$ ppm with a coupling constant of $J_{gem} = 14.4–14.5$ Hz, and the other diastereotopic proton appears as a doublet of doublets at $\delta = 3.58–3.66$ ppm with a coupling constant of $J_{gem} = 14.4–14.5$ Hz; proton H-8 is usually observed as a broad signal at $\delta = 4.66–5.22$ ppm. Finally, ^{13}C NMR, DEPT-135, and two-dimensional heteronuclear NMR spectra provided the final structural elucidation of the derivatives. The mass spectra of all compounds exhibit well-defined molecular ions with particular fragmentation patterns involving the loss of Ar–CH=CH_2. This fragmentation occurs by scission on the C–C bond in β position to the diazepine NH, followed by a C–N cleavage generating a stable cation radical with high relative intensity [6].

The formation of the pyrimidodiazepines **4**, **5**, and **7** proceeded regioselectively, despite the existence of two different amino groups in the starting 1,2-diaminopyrimidines **1** and **6**, which can also be explained as a matter of the high nucleophilicity of the amino groups on C-5 of the 1,2-diaminopyrimidines mentioned above, enhanced by the electronic effect of the pyrimidine ring and the other amino groups attached to it [7, 8]. Therefore, condensation of the amino group in C-5 with the carbonyl group in chalcones **2** and **3** can be followed by Michael's reaction of the amino group at C-4 of the C=C double bond.

Antimycobacterial screening

Mycobacterium species were obtained from the Laboratorio Micobacterias, Instituto Nacional de Salud, Bogotá, Colombia. *M. tuberculosis* H37Rv (ATCC 27294), the resistant variants (ATCC 35837 ethambutol resistant, ATCC 35838 rifampicin resistant, ATCC 35822 isoniazid resistant, and ATCC 35820 streptomycin resistant), five strains of *M. tuberculosis* Beijing genotype belonging to the Colombia National Study of Drug Resistance, and five clinical isolates

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from humans with mesotherapy-associated outbreak caused by nontuberculous mycobacteria (NTM; *M. chelonae*, *M. fortuitum*, *M. intracellulare*, *M. scrofulaceum*, and *M. szulgai*) were used. The antimycobacterial activity of the derivates dissolved in dimethylsulfoxide (DMSO; Sigma, NJ, USA) was evaluated following the MTT colorimetric microdilution protocol described by Caviedes et al. [9], with modifications. For standard tests, the minimum inhibitory concentration (MIC)

Table 1. *In vitro* activity (MIC in μg/mL) of 12 diazepine derivates against 15 *Mycobacterium* spp.

Compd	Log*P*	Dipole (*D*)	MIC (μg/mL)				
			***Mycobacterium* spp.**				
			H37Rv	**35837**	**35838**	**35822**	**35820**
4a	3.28	1.751	32	32	32	32	32
4b	2.88	1.759	>32	>32	>32	>32	>32
4c	2.72	1.785	>32	>32	>32	32	32
4d	2.60	1.678	>32	>32	>32	>32	>32
5a	3.28	1.119	32	32	32	32	32
5b	2.88	1.611	>32	>32	>32	32	32
5c	2.72	1.576	32	32	32	32	32
5d	3.64	1.430	32	32	32	32	32
7a	4.52	3.463	>32	>32	>32	>32	>32
7b	3.97	4.424	>32	>32	>32	>32	>32
7c	3.84	4.754	>32	>32	>32	>32	>32
7d	4.89	3.005	>32	>32	>32	>32	>32
Iso	–	–	0.25	0.25	0.25	>0.5	0.25
Rif	–	–	0.125	0.125	>0.5	0.125	0.125
Compd	**Log*P***	**Dipole (*D*)**	**MTB411**	**MTB985**	**MNT1407**	**MNT1073**	**MNT1100**
4a	3.28	1.751	16	16	32	>32	16
4b	2.88	1.759	>32	>32	>32	>32	>32
4c	2.72	1.785	32	32	>32	>32	>32
4d	2.60	1.678	>32	>32	>32	>32	32
5a	3.28	1.119	32	32	32	>32	32
5b	2.88	1.611	>32	>32	>32	>32	32
5c	2.72	1.576	32	32	32	>32	32
5d	3.64	1.430	32	32	32	>32	32
7a	4.52	3.463	>32	>32	>32	>32	>32
7b	3.97	4.424	>32	>32	>32	>32	>32
7c	3.84	4.754	>32	>32	>32	>32	>32
7d	4.89	3.005	>32	>32	>32	>32	>32
Iso	–	–	>0.5	>0.5	>0.5	>0.5	>0.5
Rif	–	–	>0.5	>0.5	0.125	>0.5	>0.5
Compd	**Log*P***	**Dipole (*D*)**	**MTB2556**	**MTB4000**	**UT544**	**MNT1193**	**MNT1408**
4a	3.28	1.751	16	16	16	32	>32
4b	2.88	1.759	>32	>32	>32	>32	>32
4c	2.72	1.785	32	32	32	>32	>32
4d	2.60	1.678	>32	>32	>32	>32	>32
5a	3.28	1.119	32	32	32	32	>32
5b	2.88	1.611	32	32	32	32	>32
5c	2.72	1.576	32	32	16	32	>32
5d	3.64	1.430	32	32	16	32	>32
7a	4.52	3.463	>32	>32	>32	>32	>32
7b	3.97	4.424	>32	>32	>32	>32	>32
7c	3.84	4.754	>32	>32	>32	>32	>32
7d	4.89	3.005	>32	>32	>32	>32	>32
Iso	–	–	>0.5	>0.5	>0.5	>0.5	>0.5
Rif	–	–	>0.5	>0.5	>0.5	>0.5	>0.5

M. tuberculosis H37Rv (27294) susceptible to all five first-line antituberculosis drugs (streptomycin, isoniazid, rifampicin, ethambutol, and pirazinamide), H37Rv (35837) ethambutol resistant, H37Rv (35838) rifampicin resistant, H37Rv (35822) isoniazid resistant, and H37Rv (35820) streptomycin resistant; MTB2556, MTB4000, UT544, MTB411, MTB985 (*M. tuberculosis* clinical isolates Beijing genotype, isoniazid and rifampicin resistant); MNT 1407 (*M. chelonae* clinical isolate), MNT1073 (*M. fortuitum* clinical isolate), MNT1100 (*M. szulgai* clinical isolate), MNT1193 (*M. scrofulaceum* clinical isolate), and MNT1408 (*M. intracellulare* clinical isolate).

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values of rifampicin and isoniazid (Sigma) were determined each time. The MIC of each molecule corresponds to the lowest concentration at which the bacteria tested do not show any growth. Susceptibility testing was performed three times. The results consigned in Table 1 were expressed as geometric mean (GM) of the MIC.

Four diazepine derivates (**4a**, **5a**, **5c**, and **5d**) were moderately active (good activity MIC below 10 μg/mL) [10] against 13 microorganisms; the rest inhibited the growth of *M. tuberculosis* strains. Animal studies have shown that the globally emerging Beijing genotype strains of *M. tuberculosis* are more virulent than other strains [11]. Diazepine derivates have been shown to be fatty acid biosynthesis inhibitors in other microorganisms [12]. This is an important molecular target in mycobacteria [13]. In addition, the antimicrobial activity against *Mycobacterium* species depends on how efficiently solutes cross the cell wall, which constitutes an efficient permeability barrier. Mycobacteria have a lipid-rich, hydrophobic cell wall, principally in the form of very-long-chain fatty acids (mycolic acids), which account for 30–60% of the weight of the cell wall. This cell wall obviously acts as a barrier to the penetration of hydrophilic solutes, causing that mycobacteria are often more susceptible to less polar compounds [14, 15]. Furthermore, we performed molecular modeling studies, using the CS Chem-Office Software version 9.0 (Cambridge software) [16]. All molecules were constructed using Chem Draw Ultra 9.0 and saved as template structures. For every compound, the template structure was suitably changed considering its structural features and copied to Chem 3D 9.0 to create a three-dimensional (3D) model. Finally, the model was cleaned up and subjected to energy minimization using MOPAC, with the Austin Model method. The minimization was executed until the root mean square (RMS) gradient value reached a value smaller than 0.0001 kcal/mol. The lowest energy structure was used for each molecule to calculate Log*P* and dipole values (Table 1), as lipophilicity measures [17]. The four diazepine derivates, **4a**, **5a**, **5c**, and **5d**, showed appropriate values for Log*P* and dipole, in accordance with their antimicrobial activity. In contrast, the derivatives **7a**, **7b**, **7c**, and **7d** showed the lowest lipophilicity in comparison with the other diazepines reported, and therefore less antimycobacterial activity.

Conclusion

We described herein the synthesis and antimycobacterial activity of three novel series of pyrimido[4,5-*b*]diazepines, which have a methylenedioxyaryl moiety. The new compounds were obtained in a straightforward manner and were fully characterized. The pyrimido[4,5-*b*]diazepines synthesized were tested against a wide range of *Mycobacterium* species. Four diazepine derivates (**4a**, **5a**, **5c**, and **5d**) were active against 13 microorganisms.

Experimental

Commercially available starting materials, reagents, and solvents were used as supplied. TLC analyses were performed on Merck silica gel 60 F_{254} aluminum plates. Melting points were determined in a Buechi melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were run on a Bruker AVANCE 400 spectrometer operating at 400 and 100 MHz, respectively, using DMSO-d_6 and $CDCl_3$ as solvents and tetramethylsilane as internal reference. The mass spectra were scanned on a Shimadzu GCMS-QP 2010 spectrometer (equipped with a direct inlet probe) operating at 70 eV. The elemental analyses were obtained using a Thermo Finnigan Flash EA1112 CHN (STIUJA) elemental analyzer. Microwave experiments were carried out on a focused microwave reactor (300W CEM Discover^{TM}).

*General procedure for the synthesis of 8-(benzo[d][1,3]-dioxol-5-yl)-6-aryl-8,9-dihydro-7H-pyrimido[5,4-b][1,4]-diazepine-2,4-diamines (**4a–d**) and 6-(benzo[d][1,3]dioxol-5-yl)-8-aryl-8,9-dihydro-7H-pyrimido[5,4-b][1,4]diazepine-2,4-diamines (**5a–d**)*

Compounds **4** and **5** were obtained by a mixture of the dihydrochloride of 2,4,5,6-tetraaminopyrimidine **1** (0.100 g, 0.47 mmol) and methylenedioxychalcone **2** or **3** (0.94 mmol) in methanol (30 mL) under reflux for 30 min. Reaction progress was controlled by TLC. The precipitate formed was filtrated and purified by column chromatography on silica gel by using a mixture of CH_2Cl_2/MeOH (20:1) as eluent.

*8-(Benzo[d][1,3]dioxol-5-yl)-6-(4-chlorophenyl)-8,9-dihydro-7H-pyrimido[5,4-b][1,4]diazepine-2,4-diamine (**4a**)*

This compound was obtained as a yellow solid in 93% yield. M.p. 186°C; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.13$ (dd, $J_{gem} = 14.4$ Hz, $J_{trans} = 7.6$ Hz, 1H, CH_{gem}), 3.31 (d, $J = 14.4$, 1H, CH_{gem}), 4.66 (s, 1H), 4.69 (s, 2H, NH_2), 5.51 (s, 1H, NH), 5.57 (s, 2H, NH_2), 5.93 (s, 2H), 6.74–6.75 (m, 3H), 7.29 (d, $J = 8.4$, 2H), 7.59 (d, $J = 8.4$, 2H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 41.5$, 58.5, 101.2, 102.6, 106.6, 108.4, 119.5, 127.6, 128.4, 128.5, 135.0, 136.9, 139.2, 139.3, 147.3, 148.2, 153.9, 155.7, 160.2, and 163.9 ppm; FTIR (KBr) $\nu = 3477$, 3383, 3161, 3071, 2988, 1587, 1567, and 1550 cm^{-1}; MS (EI, 70 eV), *m*/*z* (%) 410 [M+2] (29), 408 [M$^+$] (86), 393 (43), 260 (100). Anal. Calcd. for $C_{20}H_{17}ClN_6O_2$: C, 58.75; H, 4.19; N, 20.56. Found: C, 58.83; H, 4.06; N, 20.68.

*8-(Benzo[d][1,3]dioxol-5-yl)-6-(4-fluorophenyl)-8,9-dihydro-7H-pyrimido[5,4-b][1,4]diazepine-2,4-diamine (**4b**)*

This compound was obtained as a yellow solid in 87% yield. M.p. 146°C; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.13$ (dd, $J_{gem} = 14.6$ Hz, $J_{trans} = 7.2$ Hz, 1H, CH_{gem}), 3.29 (d, $J = 14.6$, 1H, CH_{gem}), 4.67 (d, $J_{trans} = 7.2$ Hz, 1H), 4.78 (s, 2H, NH_2), 5.63 (s, 1H, NH), 5.69 (s, 2H, NH_2), 5.91 (s, 2H), 6.71–6.74 (m, 3H), 7.00 (d, $J = 8.4$, 2H), 7.63 (d, $J = 8.4$, 2H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 41.5$, 58.6, 101.2, 102.5, 106.6, 108.4, 115.1,

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119.4, 128.2, 128.3, 128.5, 137.0, 147.3, 148.1, 153.8, 156.0, 160.2, 162.1, 163.9, and 164.6 ppm; FTIR (KBr) ν = 3488, 3387, 3162, 3071, 2990, 1599, 1584, and 1566 cm^{-1}; MS (EI, 70 eV), *m/z* (%) 392 [M^+] (91), 377 (46), 244 (100). Anal. Calcd. for $C_{20}H_{17}FN_6O_2$: C, 61.22; H, 4.37; N, 21.42. Found: C, 61.18; H, 4.36; N, 21.48.

*8-(Benzo[d][1,3]dioxol-5-yl)-6-phenyl-8,9-dihydro-7H-pyrimido[5,4-b][1,4]diazepine-2,4-diamine (**4c**)*

This compound was obtained as a yellow solid in 72% yield. M.p. 142°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 3.13 (dd, J_{gem} = 14.8 Hz, J_{trans} = 7.6 Hz, 1H, CH_{gem}), 3.38 (d, J = 14.8, 1H, CH_{gem}), 4.66 (d, J_{trans} = 7.6 Hz, 1H), 4.72 (s, 2H, NH_2), 5.58 (s, 1H, NH), 5.64 (s, 2H, NH_2), 5.92 (s, 2H), 6.74–6.77 (m, 3H), 7.32 (s, 1H), 7.33 (d, J = 8.4, 2H), 7.66 (d, J = 8.4, 2H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 41.6, 58.4, 101.1, 102.4, 106.6, 108.3, 119.4, 126.3, 128.2, 128.3, 128.8, 137.1, 140.8, 147.2, 148.0, 153.7, 157.0, 159.9, 160.0, and 163.8 ppm; FTIR (KBr) ν = 3470, 3385, 2968, 1608, 1583, and 1577 cm^{-1}; MS (EI, 70 eV), *m/z* (%) 374 [M^+] (100), 359 (46), 226 (75). Anal. Calcd. for $C_{20}H_{18}N_6O_2$: C, 64.16; H, 4.85; N, 22.45. Found: C, 64.20; H, 4.86; N, 22.40.

*8-(Benzo[d][1,3]dioxol-5-yl)-6-(4-methoxyphenyl)-8,9-dihydro-7H-pyrimido[5,4-b][1,4]diazepine-2,4-diamine (**4d**)*

This compound was obtained as a yellow crystal solid in 86% yield. M.p. 143°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 3.07 (dd, J_{gem} = 14.4 Hz, J_{trans} = 7.2 Hz, 1H, CH_{gem}), 3.33 (d, J_{gem} = 14.4, 1H, CH_{gem}), 3.81 (s, 3H, OCH_3), 4.65 (d, J_{trans} = 7.2 Hz, 1H), 4.72 (s, 2H, NH_2), 5.56 (s, 1H, NH), 5.62 (s, 2H, NH_2), 5.91 (s, 2H), 6.73–6.77 (m, 3H), 6.84 (d, J = 8.8, 2H), 7.63 (d, J = 8.8, 2H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 41.3, 55.3, 58.9, 101.2, 102.7, 106.7, 108.4, 113.6, 119.5, 127.9, 128.0, 133.4, 137.3, 147.2, 148.1, 153.6, 157.1, 159.7, 159.9, 160.5, and 163.7 ppm; FTIR (KBr) ν = 3475, 3385, 3178, 2961, 1599, 1584, and 1577 cm^{-1}; MS (EI, 70 eV), *m/z* (%) 404 [M^+] (100), 389 (64), 256 (50). Anal. Calcd. for $C_{21}H_{20}N_6O_3$: C, 62.37; H, 4.98; N, 20.78. Found: C, 62.43; H, 5.04; N, 20.64.

*6-(Benzo[d][1,3]dioxol-5-yl)-8-(4-chlorophenyl)-8,9-dihydro-7H-pyrimido[5,4-b][1,4]diazepine-2,4-diamine (**5a**)*

This compound was obtained as a yellow solid in 95% yield. M.p. 173°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.21 (m, 2H), 4.74 (m, 3H, H-8, NH_2), 5.30 (s, 1H, NH), 5.60 (s, 2H, NH_2), 5.96 (s, 2H), 6.69–7.26 (m, 7H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 41.0, 58.5, 101.3, 102.7, 106.6, 107.6, 120.7, 127.5, 127.6, 129.0, 133.7, 135.3, 141.4, 148.0, 148.6, 153.8, 156.5, 159.9, 160.0, and 163.7 ppm; FTIR (KBr) ν = 3485, 3381, 2900, 1586, and 1568 cm^{-1}; MS (EI, 70 eV), *m/z* (%) 410 [M+2] (30), 408 [M^+] (100), 395 (14), 393 (43), 270 (67). Anal. Calcd. for $C_{20}H_{17}ClN_6O_2$: C, 58.75; H, 4.19; N, 20.56. Found: C, 58.78; H, 4.16; N, 20.62.

*6-(Benzo[d][1,3]dioxol-5-yl)-8-(4-fluorophenyl)-8,9-dihydro-7H-pyrimido[5,4-b][1,4]diazepine-2,4-diamine (**5b**)*

This compound was obtained as a yellow solid in 92% yield. M.p. 260°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.85 (dd, J_{gem} = 14.4 Hz, J_1 = 3.6 Hz, 1H, H-7), 3.58 (dd, J_{gem} = 14.4 Hz, J_2 = 6.4 Hz, 1H, H-7), 5.01 (m, 1H, H-8), 5.38 (s, 2H, NH_2), 5.95 (s, 2H, NH_2), 5.96 (s, 2H), 6.60 (d, 1H, NH), 6.72–7.21 (m, 7H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 39.8, 58.0, 101.5, 102.3, 106.7, 107.8, 115.1, 120.9, 128.3, 128.4, 136.3, 140.7, 140.8, 147.9, 148.1, 154.8, 155.0, 155.1, 160.9, and 164.1 ppm; FTIR (KBr) ν = 3543, 3477, 3427, 3244, 3125, 1606, and 1549 cm^{-1}; MS (EI, 70 eV), *m/z* (%) 392 [M^+] (100), 377 (53), 270 (48). Anal. Calcd. for $C_{20}H_{17}FN_6O_2$: C, 61.22; H, 4.37; N, 21.42. Found: C, 61.18; H, 4.36; N, 21.48.

*6-(Benzo[d][1,3]dioxol-5-yl)-8-phenyl-8,9-dihydro-7H-pyrimido[5,4-b][1,4]diazepine-2,4-diamine (**5c**)*

This compound was obtained as a yellow solid in 70% yield. M.p. 115°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.49 (m, 1H, H-7), 3.52 (dd, J_{gem} = 14.8 Hz, J_1 = 6.0 Hz, 1H, H-7), 4.97 (m, 1H, H-8), 5.28 (s, 2H, NH_2), 5.89 (s, 2H, NH_2), 5.94 (s, 2H), 6.41 (m, 1H, NH), 6.72–7.21 (m, 8H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 41.0, 58.6, 101.5, 102.3, 106.8, 107.8, 121.0, 126.4, 127.2, 128.5, 128.6, 136.4, 144.5, 147.8, 148.1, 155.0, 155.1, 160.6, 160.7, and 164.0 ppm; FTIR (KBr) ν = 3553, 3489, 3435, 3239, 3112, 1600, and 1552 cm^{-1}; MS (EI, 70 eV), *m/z* (%) 374 [M^+] (100), 357 (42), 270 (23). Anal. Calcd. for $C_{20}H_{18}N_6O_2$: C, 64.16; H, 4.85; N, 22.45. Found: C, 64.12; H, 4.90; N, 22.47.

*6-(Benzo[d][1,3]dioxol-5-yl)-8-(4-trifluoromethylphenyl)-8,9-dihydro-7H-pyrimido[5,4-b][1,4]diazepine-2,4-diamine (**5d**)*

This compound was obtained as a yellow solid in 84% yield. M.p. 148°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.86 (dd, J_{gem} = 14.4 Hz, J_1 = 3.6 Hz, 1H, H-7), 3.66 (dd, J_{gem} = 14.5 Hz, J_2 = 6.4 Hz, 1H, H-7), 5.12 (m, 1H, H-8), 5.38 (s, 2H, NH_2), 5.93 (s, 2H, NH_2), 5.94 (s, 2H), 6.70 (d, J = 3.6, 1H, NH), 6.72–7.54 (m, 7H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 41.0, 58.3, 101.5, 102.4, 106.7, 107.8, 120.9, 125.3, 125.3, 127.3, 127.4, 136.2, 147.9, 148.1, 149.0, 154.6, 155.1, 155.2, 160.9, 164.1, and 171.3 ppm; FTIR (KBr) ν = 3474, 3387, 1587, and 1569 cm^{-1}; MS (EI, 70 eV), *m/z* (%) 442 [M^+] (100), 427 (46), 270 (35). Anal. Calcd. for $C_{21}H_{17}F_3N_6O_2$: C, 57.01; H, 3.87; N, 19.00. Found: C, 56.98; H, 4.00; N, 19.97.

*General procedure for the synthesis of 6-(benzo[d][1,3]dioxol-5-yl)-8-aryl-2-(methylthio)-8,9-dihydro-7H-pyrimido[5,4-b][1,4]diazepin-4-amines (**7a**–**d**)*

Compound **7** was obtained using microwave irradiation (120°C, 75 W, CEM Discovery) during 4–14 min, in the reaction of 2-methylthio-4,5,6-triaminopyrimidine **6** (0.60 mmol) and chalcone **3** (0.30 mmol) in DMF (0.5 mL) and a catalytic amount of $BF_3{\cdot}OEt_2$. After the reaction mixture was cooled to room temperature, ethanol (5.0 mL) was added and the precipitate formed was filtrated and purified by column chromatography on silica gel by using a mixture of CH_2Cl_2/MeOH (20:1) as eluent.

*6-(Benzo[d][1,3]dioxol-5-yl)-8-(4-chlorophenyl)-2-(methylthio)-8,9-dihydro-7H-pyrimido[5,4-b][1,4]-diazepin-4-amine (**7a**)*

This compound was obtained as a yellow solid in 95% yield. M.p. 258°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.39 (s, 3H), 2.76 (d, J_{gem} = 14.4, 1H, H-7), 3.76 (dd, J_{gem} = 14.4, J = 5.6, 1H, H-7), 5.11 (m, 1H, H-8), 5.96 (s, 2H), 6.47 (s, 2H, NH_2), 6.71–7.33 (m, 7H), 7.47 (d, J = 5.2, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 13.7, 39.3, 58.5, 101.6, 104.8, 107.1, 107.8, 121.7, 121.8, 128.3, 128.4, 131.7, 135.2, 143.1, 147.8, 148.6, 153.5, 159.2, 159.3, 162.5, and 166.2 ppm; FTIR (KBr) ν = 3513, 3432, 3118, 2978, and 1568 cm^{-1}; MS (EI, 70 eV), *m/z* (%) 441 [M+2] (41), 439 [M^+]

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(100), 426 (18), 424 (48), 301 (57). Anal. Calcd. for $C_{21}H_{18}ClN_5O_2S$: C, 57.33; H, 4.12; N, 15.92. Found: C, 57.95; H, 3.98; N, 16.04.

*6-(Benzo[d][1,3]dioxol-5-yl)-8-phenyl-2-(methylthio)-8,9-dihydro-7H-pyrimido[5,4-b][1,4]diazepin-4-amine (**7b**)*

This compound was obtained as a yellow solid in 78% yield. M.p. 260°C; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.40$ (s, 3H), 2.78 (d, $J_{gem} = 14.4$, 1H, H-7), 3.72 (dd, $J_{gem} = 14.4$, $J = 6.0$, 1H, H-7), 5.10 (m, 1H, H-8), 5.95 (s, 2H), 6.44 (s, 2H, NH_2), 6.70–7.27 (m, 8H), 7.41 (d, $J = 5.2$, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 13.7$, 39.6, 59.1, 101.6, 104.7, 107.1, 107.8, 121.7, 126.4, 127.3, 128.5, 128.6, 135.4, 144.1, 147.7, 148.5, 153.6, 159.4, 162.4, and 166.1 ppm; FTIR (KBr) $\nu = 3549$, 3402, 3183, 2956, and 1518 cm^{-1}; MS (EI, 70 eV), *m/z* (%) 405 [M$^+$] (100), 390 (50), 301 (50). Anal. Calcd. for $C_{21}H_{19}N_5O_2S$: C, 62.21; H, 4.72; N, 17.27. Found: C, 62.23; H, 4.76; N, 17.28.

*6-(Benzo[d][1,3]dioxol-5-yl)-8-(4-methoxyphenyl)-2-(methylthio)-8,9-dihydro-7H-pyrimido[5,4-b][1,4]-diazepin-4-amine (**7c**)*

This compound was obtained as a yellow solid in 83% yield. M.p. 201°C; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.41$ (s, 3H), 2.80 (d, $J_{gem} = 14.6$, 1H, H-7), 3.68 (dd, $J_{gem} = 14.6$, $J = 6.4$, 1H, H-7), 3.73 (s, 3H, OCH_3), 5.03 (m, 1H, H-8), 5.88 (s, 2H), 6.48 (s, 2H, NH_2), 6.61–7.67 (m, 7H), 7.37 (d, $J = 5.2$, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 13.7$, 39.5, 58.8, 101.2, 104.8, 107.1, 108.2, 113.8, 119.6, 128.7, 128.8, 133.4, 138.4, 146.3, 147.5, 151.6, 153.4, 159.7, 159.8, 160.5, 162.5, and 166.0 ppm; FTIR (KBr) $\nu = 3441$, 3385, 3126, 2904, and 1535 cm^{-1}; MS (EI, 70 eV), *m/z* (%) 436 [M$^+$] (18), 435 (64), 420 (46), 301 (11), 287 (100). Anal. Calcd. for $C_{22}H_{21}N_5O_3S$: C, 60.67; H, 4.86; N, 16.08. Found: C, 60.63; H, 4.80; N, 16.02.

*6-(Benzo[d][1,3]dioxol-5-yl)-8-(4-trifluorophenyl)-2-(methylthio)-8,9-dihydro-7H-pyrimido[5,4-b][1,4]-diazepin-4-amine (**7d**)*

This compound was obtained as a yellow solid in 81% yield. M.p. 215°C; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.41$ (s, 3H), 2.81 (d, $J_{gem} = 14.4$, 1H, H-7), 3.81 (dd, $J_{gem} = 14.4$, $J = 6.0$, 1H, H-7), 5.22 (m, 1H, H-8), 5.95 (s, 2H), 6.47 (s, 2H, NH_2), 6.69–7.54 (m, 7H), 7.50 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 13.7$, 39.3, 58.9, 101.6, 104.9, 107.1, 107.7, 121.7, 125.3, 125.4, 127.3, 127.4, 135.1, 147.8, 148.5, 148.6, 151.6, 153.5, 159.2, 159.3, 162.5, and 166.3 ppm; FTIR (KBr) $\nu = 3500$, 3489, 3109, 2976, and 1513 cm^{-1}; MS (EI, 70 eV), *m/z* (%) 473 [M$^+$] (100), 458 (41), 301 (37). Anal. Calcd. for $C_{22}H_{18}F_3N_5O_2S$: C, 55.81; H, 3.83; N, 14.79. Found: C, 55.83; H, 3.86; N, 14.78.

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