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Synthesis and biological evaluation of new barbituric and thiobarbituric acid fluoro analogs of benzenesulfonamides as antidiabetic and antibacterial agents

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ABSTRACT

A novel series of benzenesulfonamide derivatives of barbituric and thiobarbituric acids (**2**–**12**) were synthesized by condensation and cyclization reactions of various ureido and thioureido derivatives of sulfanilamides. Different substituents have been incorporated at C-5 of barbituric and thiobarbituric acid. Fluoro- and trifluoroacetyl substituents have been installed on various positions and their comparative biological screening was performed with the corresponding non-fluorinated analogs. The synthesized compounds were evaluated for their antimicrobial and antidiabetic activities. Some of the target compounds with fluorine substitution have shown very good antibacterial and antidiabetic activities.

1. Introduction

The barbiturates (BA) and thiobarbiturates (TBA) exert a broad range of pharmacological actions, including sedation, general anesthesia, and anticonvulsant and anxiolytic effects. They bind to specific regions of various receptors e.g. to γ-aminobutyric acid (GABA), nicotinic-acetylcholine (nAChR) or BK (big potassium) channel receptors which are all ligand-gated ion channels [1,2]. The binding to the GABA receptor requires that the C-5 atom be substituted by alkyl or aryl groups i.e. the BA and TBA are not biologically active themselves. The substitution enhances lipid solubility and facilitates transport of BA and TBA toward their enzyme targets [3]. 5-Benzylidene barbiturate and thiobarbiturate derivatives have been shown to exhibit inhibitory effect against mushroom tyrosinase and antibacterial activities against Gram-positive and Gram-negative bacteria [4]. It was also reported that the inhibitory effects of 5-benzylidene thiobarbiturates on tyrosinase were more potent than 5-benzylidene barbiturates. The position C-5 is therefore critical in enhancing the lipid solubility of the barbiturate or thiobarbiturates and hence pivotal in modulating the biological activity. Furthermore, in heterocyclic compounds fluorine incorporation on key positions plays a significant role to alter the physico-chemical and biological characteristics of these molecules [5,6]. Fluorine substitution modulates the steric and electronic parameters thereby influencing both the pharmacodynamic and pharmacokinetic properties of drugs. Recently, some of the barbituric and thiobarbituric acid analogs have been reported to show antimicrobial [7,8], antifungal [9], antiviral [10] and antitumor [11] activities. However, no report on the fluoro or trifluoromethyl substitution is found concerning this class of compound as antimicrobial and/or as antidiabetic agents. Therefore in the light of aforementioned studies we have proposed to synthesize fluoro and trifluoromethyl analogs of benzenesulfonamide derivatives of barbituric and thiobarbituric acids along with alkyl and aryl substitutions on the position-5. The present proposal involves the synthesis of a novel series of benzenesulfonamides of barbituric and thiobarbituric acids **2**–**12** (Scheme 1). The biological profiles of fluorinated BA and TBA were compared with non-fluorinated analogs.

2. Results and discussion

2.1. Chemistry

Some new thiobarbituric and barbituric acids with incorporation of sulfa drugs and fluorine atom as the target for chemical modification to enhance their biocidal effects were synthesized. Thus, addition of aryl isothiocyanates and isocyanates to some sulfa drugs **1a**–**d** in warming DMF yielded N,N′-disubistituted thiourea and urea derivatives **2** and **3** respectively. Their IR spectra showed beside the absorption bands corresponding to the NH group at 3216–3288 cm^{-1}, two bands at 1345–1372 cm^{-1} and 1177–1190 cm^{-1} for the SO_2N function, in addition to a characteristic C=S band at 1155–1165 cm^{-1} and a urea carbonyl absorption at 1645–1665 cm^{-1} for compounds **2** and **3** respectively. Following the literature method [12], heterocyclization of the

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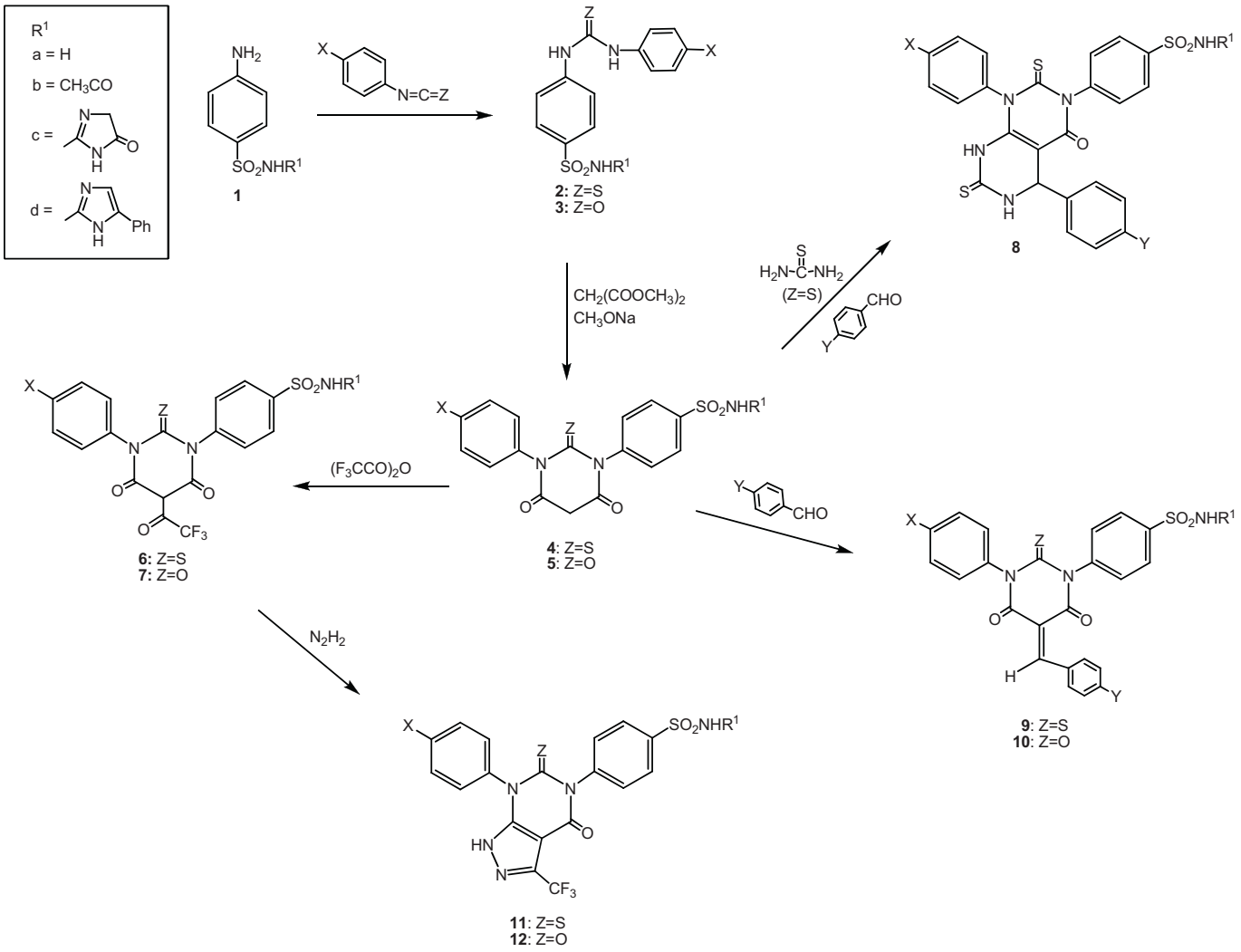

Scheme 1. Synthesis of thiobarbiturates and their trifluoromethyl analogs.

thiourea and urea derivatives **2** and **3** via refluxing with malonic acid in acetyl chloride led to the direct formation of thiobarbituric **4** and barbituric **5** acids respectively. Fluorination of compounds **4** and **5** by treatment with trifluoroacetic anhydride in THF afforded the 5-trifluoroacetyl derivatives **6** and **7** respectively. The IR spectra of the thiobarbituric acid derivatives **4** exhibited absorption bands at 1140–1152 cm^{-1} and 1668–1678 cm^{-1} for the C=S and C=O functional groups respectively. The barbituric acids **5**, however, exhibited only the carbonyl absorption at 1669–1685 cm^{-1}. On the other hand, the IR spectra of the pyrimidine derivatives **5** and **7** were characterized by the appearance of a new band at 1705–1715 cm^{-1} attributed to the $COCF_3$ group. The structures of the compounds **2**–**7** were further supported by elemental analysis (Table 1) and their ^1H NMR and ^{13}C NMR data (Table 2).

In 1893, the Italian chemist Pietro Biginelli reported the acid-catalyzed cyclocondensation reaction of ethyl acetoacetate, benzaldehyde and urea [13]. The reaction was performed simply by heating a mixture of the three components dissolved in ethanol with a catalytic amount of HCl at reflux temperature. The product of this novel one-pot, three-component synthesis that precipitated on cooling of the reaction mixture was identified correctly by Biginelli as 3,4-dihydropyrimidin-2(1*H*)-one (DHPM). Following this protocol, the target molecules, 1,3,5-trisubistitute-2,7-dithioxo-octahydropyrimido[4,5-d]pyrimidin-4-ones (**8**) were synthesized in good yield by the one pot reaction of thiobarbituric acids **4**, aromatic aldehydes and thiourea in refluxing ethanol using few drops of concentrated hydrochloric acid as catalyst [8,14,15]. Their IR spectra showed beside the absorption bands corresponding to the NH group at 3222–3288 cm^{-1}, two thiocarbonyl absorptions at 1155–1165 cm^{-1} and 1077–1080 cm^{-1} for the two C=S groups. The structures of the above compound **8** were further supported by their ^1H NMR and ^{13}C NMR data (Table 2). Furthermore, condensation of the pyrimidine derivatives **4** and **5** with aromatic aldehydes afforded the corresponding arylidine derivatives **9** and **10**. The ^1H NMR spectra of these arylidines are characterized by a singlet of one proton intensity at δ8.08–8.32 ppm attributed to the CH= proton. Finally, cyclization of the fluoropyrimidines **6** and **7** with hydrazine hydrate afforded the pyrazole–pyrimidine derivatives **11** and **12**. The structures of the pyrimidine derivatives **9**–**12** were supported by elemental analysis (Table 1) and their ^1H NMR and ^{13}C NMR data (Table 2).

2.2. Biological evaluation

2.2.1. In vitro antibacterial and antifungal activities

The antibacterial activity of the synthesized compounds revealed that twenty-six out of the tested forty five compounds displayed variable inhibitory effects on the growth of the tested Gram positive and Gram negative bacterial strains. In general, most

Table 1
Characterization data of compounds **2**–**12**.

Compound no.	Y	X	Yield (%)	m.p. (°C)	Mol. formula	Calc.%			Found%		
						C	H	N	C	H	N
2a$_1$		H	80	180–181	$C_{13}H_{13}N_3O_2S_2$	50.79	4.26	13.67	50.81	4.17	13.76
2a$_2$		Cl	82	174–176	$C_{13}H_{12}ClN_3O_2S_2$	45.68	3.54	12.29	45.77	3.52	12.31
2b$_1$		H	78	148–150	$C_{15}H_{15}N_3O_3S_2$	51.56	4.33	12.03	51.67	4.38	12.15
2b$_2$		Cl	76	138–140	$C_{15}H_{14}ClN_3O_3S_2$	46.93	3.68	10.95	47.01	3.72	11.02
2c		H	74	150–152	$C_{16}H_{15}N_5O_3S_2$	49.34	3.88	17.98	49.34	3.76	18.13
2d$_1$		H	76	158–160	$C_{22}H_{19}N_5O_2S_2$	58.78	4.26	15.58	58.86	4.18	15.61
2d$_2$		Cl	72	130–132	$C_{22}H_{18}ClN_5O_2S_2$	54.59	3.75	14.47	54.61	3.68	14.55
3a$_1$		H	81	220–21	$C_{13}H_{13}N_3O_3S$	53.60	4.50	14.42	53.62	4.60	14.32
3a$_2$		Cl	85	248–50	$C_{13}H_{12}ClN_3O_3S$	47.93	3.71	12.90	48.04	3.82	13.01
3b$_1$		H	79	240–242	$C_{15}H_{15}N_3O_4S$	54.04	4.54	12.60	54.18	4.66	12.77
3b$_2$		Cl	80	257–59	$C_{15}H_{14}ClN_3O_4S$	48.98	3.84	11.42	49.10	3.90	11.58
3c		Cl	72	266–268	$C_{16}H_{14}ClN_5O_4S$	47.12	3.46	17.17	47.22	3.58	17.21
3d		Cl	74	228–230	$C_{22}H_{18}ClN_5O_3S$	56.47	3.88	14.97	56.58	3.98	15.12
4a$_1$		H	70	181–182	$C_{16}H_{13}N_3O_4S_2$	51.19	3.49	11.19	51.23	3.51	11.24
4a$_2$		Cl	74	190–192	$C_{16}H_{12}ClN_3O_4S_2$	46.89	2.95	10.25	46.97	2.88	10.36
4b$_1$		H	76	130–134	$C_{18}H_{15}N_3O_5S_2$	51.79	3.62	10.07	51.81	3.71	10.18
4b$_2$		Cl	75	158–159	$C_{18}H_{14}ClN_3O_5S_2$	47.84	3.12	9.30	47.82	3.22	9.35
4c		H	68	192–194	$C_{19}H_{15}N_5O_5S_2$	49.88	3.30	15.31	49.76	3.29	15.42
4d$_1$		H	76	208–210	$C_{25}H_{19}N_5O_4S_2$	58.01	3.70	13.53	58.14	3.68	13.61
4d$_2$		Cl	72	168–170	$C_{25}H_{18}ClN_5O_4S_2$	54.39	3.29	12.69	54.40	3.21	12.73
5a$_1$		H	77	226–227	$C_{16}H_{13}N_3O_5S$	53.48	3.65	11.69	53.51	3.77	11.70
5a$_2$		Cl	76	272–274	$C_{16}H_{12}ClN_3O_5S$	48.80	3.07	10.67	48.92	3.14	10.78
5b$_1$		H	72	235–236	$C_{18}H_{15}N_3O_6S$	53.86	3.77	10.47	53.76	3.84	10.58
5b$_2$		Cl	71	276–278	$C_{18}H_{14}ClN_3O_6S$	49.60	3.24	9.64	49.55	3.35	9.75
5c		Cl	70	244–246	$C_{19}H_{14}ClN_5O_6S$	47.96	2.97	14.72	48.12	3.01	14.84
5d		Cl	72	216–218	$C_{25}H_{18}ClN_5O_5S$	56.02	3.39	13.07	56.14	3.47	13.22
6a$_1$		H	69	215–216	$C_{18}H_{12}F_3N_3O_5S_2$	45.86	2.57	8.91	45.92	2.63	9.04
6a$_2$		Cl	72	200–202	$C_{18}H_{11}ClF_3N_3O_5S_2$	42.74	2.19	8.31	42.63	2.08	8.28
6b$_1$		H	68	140–143	$C_{20}H_{14}F_3N_3O_6S_2$	46.78	2.75	8.18	46.87	2.74	8.21
6b$_2$		Cl	70	296–298	$C_{20}H_{13}ClF_3N_3O_6S_2$	43.84	2.39	7.67	43.90	2.43	7.75
6c		H	64	338–340	$C_{21}H_{14}F_3N_5O_6S_2$	45.57	2.55	12.65	45.64	2.62	12.58
6d$_1$		H	72	283–285	$C_{27}H_{18}F_3N_5O_5S_2$	52.85	2.96	11.41	52.91	2.87	11.32
6d$_2$		Cl	76	228–230	$C_{27}H_{17}ClF_3N_5O_5S_2$	50.04	2.64	10.81	50.12	2.58	10.93
7a$_1$		H	68	188–190	$C_{18}H_{12}F_3N_3O_6S$	47.48	2.66	9.23	47.51	3.76	9.41
7a$_2$		Cl	69	212–214	$C_{18}H_{11}ClF_3N_3O_6S$	44.14	2.26	8.58	44.28	2.36	8.60
7b$_1$		H	65	240–242	$C_{20}H_{14}F_3N_3O_7S$	48.29	2.84	8.45	48.33	2.80	8.58
7b$_2$		Cl	68	190–192	$C_{20}H_{13}ClF_3N_3O_7S$	45.17	2.46	7.90	45.20	2.56	8.11
8a$_1$	H	H	66	170–172	$C_{24}H_{19}N_5O_3S_3$	55.26	3.67	13.43	55.21	3.77	13.48
8a$_2$	2,4-diF	H	64	166–167	$C_{24}H_{17}F_2N_5O_3S_3$	51.69	3.07	12.56	51.64	3.05	12.64
8a$_3$	H	Cl	67	154–156	$C_{24}H_{18}ClN_5O_3S_3$	51.84	3.26	12.55	51.80	3.28	12.64
8a$_4$	4-Br	Cl	69	160–162	$C_{24}H_{17}BrClN_5O_3S_3$	45.40	2.70	11.03	45.31	3.04	11.00
8a$_5$	2,4-diF	Cl	66	146–148	$C_{24}H_{16}ClF_2N_5O_3S_3$	48.69	2.72	11.83	48.62	2.82	11.88
9a$_1$	H	H	80	192–193	$C_{23}H_{17}N_3O_4S_2$	59.60	3.70	9.07	59.64	3.80	8.98
9a$_2$	2,4-diF	H	81	198–199	$C_{23}H_{15}F_2N_3O_4S_2$	55.30	3.03	8.41	55.51	3.09	8.38
9a$_3$	H	Cl	82	176–178	$C_{23}H_{16}ClN_3O_4S_2$	55.47	3.24	8.44	55.56	3.28	4.82
9a$_4$	2,4-diF	Cl	78	188–190	$C_{23}H_{14}ClF_2N_3O_4S_2$	51.74	2.64	7.87	51.88	2.71	7.94
10a$_1$	H	H	79	206–208	$C_{23}H_{17}N_3O_5S$	61.74	3.83	9.39	61.82	3.94	9.42
10a$_2$	2,4-diF	H	77	230–232	$C_{23}H_{15}F_2N_3O_5S$	57.14	3.13	8.69	57.25	3.18	8.76
10a$_3$	H	Cl	82	260–262	$C_{23}H_{16}ClN_3O_5S$	57.32	3.35	8.72	57.23	3.40	8.77
10a$_4$	4-CH_3O	Cl	78	250–252	$C_{24}H_{18}ClN_3O_6S$	56.31	3.54	8.21	56.38	3.62	8.14
10a$_5$	4-Cl	Cl	79	245–248	$C_{23}H_{15}Cl_2N_3O_5S$	53.50	2.93	8.14	53.61	3.04	8.20
10a$_6$	2,4-diF	Cl	74	250–252	$C_{23}H_{14}ClF_2N_3O_5S$	53.34	2.72	8.11	53.48	2.84	8.09
10b	2,4-diF	H	76	240–242	$C_{25}H_{17}F_2N_3O_6S$	57.14	3.26	8.00	57.22	3.31	7.98
10c	2,4-diF	Cl	72	261–262	$C_{26}H_{16}ClF_2N_5O_6S$	52.05	2.69	11.67	52.13	2.70	11.76
10d$_1$	H	Cl	73	257–258	$C_{32}H_{22}ClN_5O_5S$	61.59	3.55	11.22	61.67	3.54	11.31
10d$_2$	2,4-diF	Cl	72	250–252	$C_{32}H_{20}ClF_2N_5O_5S$	58.23	3.05	10.61	58.14	3.12	10.74
11a$_1$		H	67	282–284	$C_{18}H_{12}F_3N_5O_3S_2$	46.25	2.59	14.98	46.35	2.61	15.02
11a$_2$		Cl	69	220–222	$C_{18}H_{11}ClF_3N_5O_3S_2$	43.08	2.21	13.95	43.12	2.34	14.15
11d$_1$		H	65	224–226	$C_{27}H_{18}F_3N_7O_3S_2$	53.30	2.98	16.08	53.28	3.04	16.20
11d$_2$		Cl	64	208–210	$C_{27}H_{17}ClF_3N_7O_3S_2$	50.35	2.66	15.22	50.46	2.78	15.21
12a$_1$		H	66	200–202	$C_{18}H_{12}F_3N_5O_4S$	47.90	2.68	15.52	48.11	2.76	15.64
12a$_2$		Cl	67	256–258	$C_{18}H_{11}ClF_3N_5O_4S$	44.50	2.28	14.42	44.45	2.34	14.33
12b		Cl	64	269–270	$C_{20}H_{14}F_3N_5O_5S$	48.68	2.86	14.19	48.70	2.91	14.24
12d		Cl	65	280–282	$C_{27}H_{17}ClF_3N_7O_4S$	51.64	2.73	15.61	51.71	2.83	15.78

of the compounds showed better activity profile against the Gram positive rather than the Gram negative bacteria. Among the Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* showed relatively high sensitivity toward the tested compounds. In this view, compounds **6c** and **10a$_2$** were equipotent to Ampicillin (MIC 6.25 μg/mL) against *S. aureus*, whereas the analogs **6a$_2$**, **6b$_2$**, **6d$_2$**, **7a$_1$**, **7a$_2$**, **9a$_4$**, and **10c** (MIC 12.5 μg/mL) were 50% less active than Ampicillin. Furthermore, compounds **4c**, **4d$_1$**, **4d$_2$**, **5c**, **8a$_2$**, **8a$_5$**, **9a$_2$**, **10a$_6$**, **10b**, **10d$_2$** and **11d$_2$** (MIC 25 μg/mL) showed 25% of the activity of Ampicillin against the same organism. With regard to the activity against *B. subtilis*, the best activity was displayed by compounds **6c**, **6d$_2$**, **10a$_2$** and **10c** which were equipotent to Ampicillin (MIC 12.5 μg/mL), whereas the analogs **4a$_2$**, **4d$_2$**, **5c**, **6a$_2$**, **8a$_5$**, **9a$_4$**, **10b** and **11d$_1$** (MIC 25 μg/mL) which represented half the

Table 2
^{1}H NMR and ^{13}C NMR Spectral data (δ/ppm) of compounds **2–12.**

Compound no.	Y	X	^{1}H NMR			^{13}C NMR		
			Ar–*H* (*m*)	N*H*$_2$ or N*H*	Other *H*	Ar–*C*	*C*O or *C*S	Other *C*
2a$_1$		H	6.74–7.82 (9H)	8.06, 8.19, 8.34				
2a$_2$		Cl	6.62–7.79 (8H)	8.12, 8.25, 8.38		120.7, 121.8, 125.6, 129.0, 129.6, 134.9,138.7, 144.0	180.2	
2b$_1$		H	6.81–7.84 (9H)	8.00, 8.13, 8.24	2.14 (*s*, CH_3)	120.4, 120.7, 124.1, 125.5, 128.5, 134.3, 140.8, 144.6	173.1, 179.4	16.4 (CH_3)
2b$_2$		Cl	6.78–7.90 (8H)	8.10, 8.24, 8.35	2.20 (*s*, CH_3)			
2c		H	6.62–7.79 (9H)	7.89, 8.21, 8.92	2.31 (*s*, CH_2)			
2d$_1$		H	6.82–7.68 (15H)	7.92, 8.31				
2d$_2$		Cl	6.76–7.91 (14H)	8.03, 8.14		120.4, 121.6, 122.2, 122.8, 124.6, 125.2, 127.1, 127.7, 128.6, 129.1, 129.4, 133.6, 136.7, 136.9, 140.4	181.3	
3a$_1$		H	7.00–7.85 (9H)	7.98, 8.26		120.4, 120.7, 124.1, 125.8, 128.6, 134.5, 138.2, 141.2	156.3	
3a$_2$		Cl	7.25–7.92 (8H)	8.14, 8.43		120.6, 121.8, 125.6, 129.2, 129.4, 134.9, 136.3, 142.0	158.9	
3b$_1$		H	7.13–7.82 (9H)	8.05, 8.24, 8.64	2.02 (*s*, CH_3)	119.3, 120.2, 122.3, 126.7, 129.4, 133.6, 137.9, 140.2	155.4, 173.1	16.6 (CH_3)
3b$_2$		Cl	7.20–7.79 (8H)	7.98, 8.34, 8.58	2.14 (*s*, CH_3)	118.4, 121.3, 124.5, 125.2, 127.7, 132.9, 136.5, 144.2	154.2, 172.8	16.2 (CH_3)
3c		Cl	7.26–7.90 (8H)	8.10, 8.34, 8.42	2.21 (*s*, CH_2)	120.9, 121.2, 123.8, 125.4, 129.0, 134.2, 138.7, 141.6, 163.2		
3d		Cl	7.10–7.74 (14H)	7.90, 8.21, 13.4		121.3, 122.2, 123.4, 125.6, 125.9, 127.4, 128.5, 129.1, 130.0, 131.2, 134.2, 136.4, 137.2, 138.8, 140.9	154.6	
4a$_1$		H	7.02–7.91 (9H)	8.02	3.17 (*s*, CH_2)	120.4, 120.7, 124.1, 125.7, 128.6, 134.9, 140.8, 144.0	168.1, 169.3, 177.8	34.5 (CH_2)
4a$_2$		Cl	7.23–7.89 (8H)	8.13	3.20 (*s*, CH_2)			
4b$_1$		H	7.10–7.92 (9H)	8.09	2.06 (*s*, CH_3), 3.19 (*s*, CH_2)	120.3, 121.0, 124.2, 125.8, 127.9, 133.4, 141.2, 143.8	168.2, 168.9, 173.3, 178.1	16.6 (CH_3), 35.1 (CH_2)
4b$_2$		Cl	7.16–7.90 (8H)	8.18	2.13 (*s*, CH_3), 3.42 (*s*, CH_2)	120.7, 121.8, 125.6, 129.3, 129.5, 134.8, 138.9, 143.2	169.1, 169.6, 173.8,178.6	16.8 (CH_3), 34.9 (CH_2)
4c		H	6.98–7.82 (9H)	8.24	2.28 (*s*, CH_2), 3.30 (*s*, CH_2)	120.1, 120.8, 124.6, 126.0, 128.8, 133.8, 139.9, 142.4, 163.2	168.2, 168.9, 173.6	34.4 (CH_2), 53.1 (CH_2)
4d$_1$		H	7.00–7.91 (15H)	8.34, 12.88	3.56 (*s*, CH_2)			
4d$_2$		Cl	7.12–7.80 (14H)	8.29, 13.12	3.62 (*s*, CH_2)	120.3, 121.8, 122.2, 123.3, 124.0, 125.5, 127.1, 127.6, 128.5, 129.0, 129.4, 133.9, 136.5, 136.9, 140.1	165.4, 169.2, 177.9	35.0 (CH_2)
5a$_1$		H	7.09–7.85 (9H)	8.02	3.53 (*s*, CH_2)			
5a$_2$		Cl	7.31–7.92 (8H)	8.21	3.61 (*s*, CH_2)			
5b$_1$		H	7.06–7.86 (9H)	8.38	2.14 (*s*, CH_3), 3.54 (*s*, CH_2)			
5c		Cl	7.25–7.91 (9H)	8.09	2.26 (*s*, CH_2), 3.50 (*s*, CH_2)			
5d		Cl	7.23–7.84 (14H)	8.10, 13.20	3.55 (*s*, CH_2)			
6a$_1$		H	7.08–7.98 (8H)	8.12	4.26 (*s*, CH)			
6a$_2$		Cl	7.10–7.81 (8H)	8.26	4.18 (*s*, CH)	120.7, 121.8, 125.6, 129.1, 129.4, 134.9, 138.7, 144.3	168.3, 169.4, 178.1, 206.0	43.4 (CH), 127.8 (CF_3)
6b$_1$		H	7.13–7.90 (9H)	8.18	2.13 (*s*, CH_3), 4.24 (*s*, CH)			
6c		H	6.99–7.87 (9H)	8.20	2.19 (*s*, CH_2), 4.19 (*s*, CH)			
6d$_1$		H	7.01–7.92 (15H)	8.15	4.28 (*s*, CH)	120.2, 121.6, 122.4, 123.2, 124.4, 125.7, 127.2, 127.6, 128.5, 129.1, 129.6, 134.0, 136.3, 137.1, 140.2	167.9, 168.2, 172.9, 204.3	43.6 (CH), 128.0 (CF_3)
7a$_1$		H	7.01–7.92 (9H)	8.02	4.30 (*s*, CH)			
7a$_2$		Cl	7.26–7.90 (8H)	8.21	4.18 (*s*, CH)	119.2, 120.9, 124.8, 128.9, 129.3, 133.1, 137.6, 142.4	153.1, 167.9, 168.2, 177.3	42.2 (CH), 127.8 (CF_3)
7b$_1$		H	7.00–7.79 (9H)		2.23 (*s*, CH_3), 4.09 (*s*, CH)	120.1, 121.3, 125.8, 129.2, 129.7, 135.1, 139.4, 144.1	151.9, 168.4, 169.2, 176.4	16.8 (CH_3), 40.9 (CH), 127.1 (CF_3)
7b$_2$		Cl	7.14–7.90 (8H)		2.17 (*s*, CH_3), 4.12 (*s*, CH)			
8a$_1$	H	H	6.43–7.92 (16H)[a]		4.32 (*s*, CH), 5.12 (*brs*, NH)	120.7, 124.5, 125.3, 125.7, 125.8, 128.2, 128.5, 128.9, 133.9, 138.2, 139.6, 144.4	176.2 (CO), 179.8 (CS), 184.4 (CS)	52.9, 53.8, 73.4 (3CH)
8a$_2$	2,4-diF	H	6.74–7.93 (14H)[a]		4.40 (*s*, CH), 5.12 (*brs*, NH)	117.2, 118.4, 120.7, 124.9, 125.3, 125.9, 126.6, 129.3, 134.4, 135.9, 137.4, 143.3, 145.9, 148.8		
8a$_3$	H	Cl	6.64–7.88 (15H)[a]		4.34 (*s*, CH), 5.04 (*brs*, NH)			

Table 2 (*Continued*)

Compound no.	Y	X	^{1}H NMR			^{13}C NMR		
			Ar–*H* (*m*)	N*H*$_2$ or N*H*	Other *H*	Ar–*C*	*C*O or *C*S	Other *C*
8a$_4$	4-Br	Cl	6.74–7.79 (14H)[a]		4.18 (*s*, CH), 5.07 (*brs*, NH)	120.4, 121.3, 125.2, 125.6, 126.1, 127.7, 128.1, 128.6, 130.5, 131.8, 137.9, 144.4	175.8 (CO), 178.7 (CS), 183.9 (CS)	53.7, 54.2, 72.8 (3CH)
8a$_5$	2,4-diF	Cl	6.81–7.90 (13H)[a]		4.20 (*s*, CH), 5.13 (*brs*, NH)	116.9, 117.4, 120.8, 125.6, 125.8, 126.7, 129.4, 133.7, 136.2, 137.4, 142.9, 146.3, 149.0	177.1 (CO), 178.4 (CS), 187.1 (CS)	52.8, 53.2, 73.8 (3CH)
9a$_1$	H	H	7.01–7.89 (16H)[b]		8.03 (*s*, CH=)	120.2, 120.7, 124.3, 125.7, 126.3, 127.2, 127.6, 128.2, 128.4, 128.8, 134.7, 135.0, 138.8, 141.5, 147.4	162.7 (CO), 163.1 (CO), 182.3 (CS)	147.4 (CH=)
9a$_2$	2,4-diF	H	6.99–7.78 (14H)[b]		8.17 (*s*, CH=)	114.8, 117.2, 120.7, 121.8, 123.5, 125.5, 129.1, 129.4, 133.1, 134.9, 136.3, 141.2, 148.3	163.8 (CO), 164.4 (CO), 180.9 (CS)	149.0 (CH=)
9a$_3$	H	Cl	7.25–7.90 (15H)[b]		8.08 (*s*, CH=)			
9a$_4$	2,4-diF	Cl	6.90–7.92 (13H)[b]		8.24 (*s*, CH=)			
10a$_1$	H	H	6.92–7.92 (16H)[b]		8.32 (*s*, CH=)	119.8, 120.5, 124.8, 125.6, 126.3, 127.0, 127.7, 128.4, 128.5, 134.3, 134.8, 137.9, 141.3	150.2 (CO), 164.3 (CO), 164.8 (CO)	148.4 (CH=)
10a$_2$	2,4-diF	H	6.80–7.89 (14H)[b]		8.25 (*s*, CH=)	115.4, 116.9, 120.7, 121.2, 123.8, 125.9, 129.1, 129.5, 134.4, 134.7, 137.1, 144.3, 147.4	152.1 (CO), 161.4 (CO), 162.3 (CO)	148.9 (CH=)
10a$_3$	H	Cl	7.12–7.91 (15H)[b]		8.19 (*s*, CH=)			
10a$_4$	4–CH_3O	Cl	6.72–7.89 (14H)[b]		3.72 (*s*, OCH_3), 8.01 (*s*, C=H)			
10a$_5$	4-Cl	Cl	7.25–7.89 (14H)[b]		8.28 (*s*, CH=)	117.8, 119.2, 120.3, 125.1, 125.8, 126.4, 127.7, 127.8, 128.5, 128.7, 133.9, 134.4, 136.8, 142.4	150.8 (CO), 162.4 (CO), 163.5 (CO)	147.4 (CH=)
10a$_6$	2,4-diF	Cl	6.89–7.90 (13H)[b]		8.40 (*s*, CH=)			
10c	2,4-diF	Cl	6.92–7.92 (11H)[c]	8.32	2.05 (*s*, CH_2), 8.01 (*s*, CH=)	120.1, 121.4, 123.8, 125.6, 126.7, 128.2, 128.9, 129.1, 132.4, 135.1, 138.9, 140.4, 160.4	153.1 (CO), 164.6 (CO), 172.8 (CO)	148.8 (CH=)
10d$_1$	H	Cl	7.01–7.90 (19H)[c]	13.1	8.11 (*s*, CH=)			
10d$_2$	2,4-diF	Cl	6.87–7.91 (17H)[c]	13.4	8.09 (*s*, CH=)			
11a$_1$		H	6.98–7.91 (11H)[b]	11.68		120.4, 121.1, 123.8, 125.6, 128.4, 134.2, 137.9, 141.9	161.3 (CO), 179.4 (CS),	164.0 and 164.6 (2C=N), 113.4 (CF_3)
11a$_2$		Cl	7.23–7.86 (10H)[b]	11.74		119.2, 120.9, 124.4, 126.1, 127.9, 133.7, 138.8, 142.1	160.5 (CO), 184.2 (CS)	163.8 and 164.9 (2C=N), 114.8 (CF_3)
11d$_1$		H	7.18–7.92 (15H)[c]	11.0				
12a$_1$		H	7.00–7.76 (11H)[b]	12.3		118.8, 120.7, 123.9, 125.1, 127.6, 132.8, 136.8, 140.9	163.1 (CO), 185.6 (CS)	164.8 and 164.9 (2C=N), 114.1 (CF_3)
12b		Cl	7.00–7.94 (8H)	8.1, 11.98	2.15 (*s*, CH_3)	120.4, 120.7, 124.1, 125.5, 128.9, 134.7, 138.2, 141.4	162.4 (CO), 173.4 (CO), 183.3 (CS)	16.5 (CH_3), 164.0, 164.7 (2C=N), 112.8 (CF_3)
12d		Cl	7.28–7.84 (14H)[c]	13.5, 12.5		120.1, 120.8, 125.0, 125.8, 127.6, 128.4, 129.9, 133.9, 137.4, 138.2, 139.4, 140.6	161.8 (CO), 182.0 (CS)	165.2, 165.6 (2C=N), 115.2 (CF_3)

s, singlet; brs, broad singlet.

[a] Ar-H and 2 NH.

[b] Ar-H and NH_2.

[c] Ar-H and NH.

Table 3
In vitro antimicrobial and antifungal activities of the target compounds **2–12**.

Compound no.	*Staphylococcus aureus*		*Bacillus subtilis*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Aspergillus niger*		*Candida albicans*	
	IZ[a]	MIC[b]	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
2a$_1$	6	–	7	–	5	–	6	–	5	–	7	–
2c	8	–	5	–	10	100	7	–	9	–	9	–
3a	5	–	6	–	7	–	6	–	8	–	8	–
3c	9	–	8	–	9	–	5	–	11	100	10	100
4a$_1$	22	50	22	50	21	50	20	100	19	100	21	100
4a$_2$	21	50	26	25	22	50	22	50	20	100	24	50
4c	25	25	24	50	26	25	24	25	24	50	26	25
4d$_1$	24	25	23	50	20	50	21	50	16	100	21	50
4d$_2$	25	25	27	25	26	25	25	25	8	–	24	25
5a$_1$	21	100	20	100	19	100	16	100	14	100	17	100
5a$_2$	22	50	19	100	20	100	19	100	7	–	16	100
5c	24	25	26	25	24	50	23	50	5	–	26	25
5d	27	25	25	50	24	25	22	50	6	–	24	25
6a$_2$	26	12.5	27	25	25	25	24	50	9	–	25	25
6b$_2$	27	12.5	22	50	23	50	23	50	18	100	23	50
6c	30	6.25	28	12.5	27	12.5	26	12.5	20	100	27	25
6d$_1$	25	25	24	25	21	50	22	50	19	100	26	12.5
6d$_2$	29	12.5	26	12.5	29	6.25	25	25	21	50	25	25
7a$_1$	26	12.5	23	50	22	50	22	50	16	100	23	50
7a$_2$	25	12.5	20	100	19	100	20	100	15	100	9	–
7b$_1$	22	50	19	100	22	50	8	–	19	100	23	50
8a$_1$	16	100	18	100	9	–	6	–	–	100	7	–
8a$_2$	24	25	24	50	23	50	14	100	18	100	20	100
8a$_3$	15	100	17	100	8	–	6	–	–	–	7	–
8a$_5$	24	25	25	25	26	25	9	–	16	100	25	25
9a$_1$	20	100	21	100	20	100	21	100	5	–	19	100
9a$_2$	25	25	24	50	24	25	22	50	8	–	24	25
9a$_3$	23	50	20	100	22	50	18	100	7	–	20	100
9a$_4$	27	12.5	26	25	25	25	23	50	10	100	26	25
10a$_1$	22	50	20	100	22	50	19	100	6	–	22	50
10a$_2$	30	6.25	29	12.5	28	12.5	22	50	9	–	27	12.5
10a$_3$	17	100	16	100	12	100	12	100	8	–	19	100
10a$_6$	26	25	24	50	22	50	22	50	20	100	25	25
10b	26	25	25	25	24	25	21	100	12	100	22	50
10c	27	12.5	28	12.5	26	12.5	23	50	23	50	24	25
10d$_1$	21	50	20	100	19	100	9	–	21	50	22	50
10d$_2$	24	25	23	50	24	25	20	100	17	100	27	12.5
11a$_1$	20	50	21	50	20	50	16	100	9	–	22	50
11a$_2$	21	50	23	50	19	100	19	100	10	100	24	25
11d$_1$	22	50	24	25	22	50	23	50	11	100	28	12.5
11d$_2$	25	25	21	50	22	50	25	25	8	–	21	50
12a$_1$	20	100	19	100	21	100	18	100	9	–	26	12.5
12a$_2$	22	50	23	50	21	50	20	100	7	–	24	25
12b	21	100	20	100	24	25	22	50	6	–	23	25
12d	23	50	22	50	23	50	24	50	10	100	26	12.5
A[d]	30	6.25	30	12.5	29	6.25	28	12.5	–	–	–	–
C[e]	–	–	–	–	–	–	–	–	33	12.5	32	6.25

[a] IZ: inhibition zone.
[b] MIC: minimum inhibitory concentration.
[c] (–): inactive, MIC $\geq$ 200 μg/mL.
[d] A: Ampicillin trihydrate.
[e] C: Clotrimazole.

potency of Ampicillin. The analogs **4a$_1$**, **4c**, **4d$_1$**, **5d**, **7a$_1$**, **8a$_2$**, **9a$_2$**, **10a$_6$**, **10d$_2$**, **11a$_1$**, **11a$_2$**, **12a$_2$**, and **12d** (MIC 50 μg/mL) exhibited 25% of the potency of Ampicillin against the same species.

On the other hand, investigation of the antibacterial potency against the three tested Gram negative strains revealed that most of the active compounds showed better growth inhibitory activity against *Escherichia coli* when compared with *Pseudomonas aeruginosa*. Compound **6d$_2$** was equipotent to Ampicillin (MIC 6.25 μg/mL), whereas the analogs **6c**, **10a$_2$** and **10c** produced noticeable growth inhibitory activity (MIC 12.5 μg/mL) which was 50% of the activity of Ampicillin. Moreover, compounds **4c**, **4d$_2$**, **5d**, **6a$_2$**, **8a$_5$**, **9a$_2$**, **9a$_4$**, **10b**, **10d$_2$** and **12b** (MIC 25 μg/mL), exhibited moderate activity against the same organism. Meanwhile, the tested *P. aeruginosa* and *K. pneumonia* strains proved to be weakly sensitive to most of the tested compounds. Among these, compounds **4c**, **4d$_2$**, **6c**, **6d$_2$** and **11d$_2$** showed moderate growth inhibitory profile against *P. aeruginosa* (MIC 25 μg/mL), which was about 50% of the activity of Ampicillin (MIC 12.5 μg/mL).

Concerning the antifungal activity of the tested compounds, the results revealed that except compounds **2a$_1$**, **2c**, **3a**, **7a$_2$**, **8a$_1$** and **8a$_3$** all other tested compounds were able to produce appreciable growth inhibitory activity against *Candida albicans* (MIC values 12.5–100 μg/mL, respectively) when compared to Clotrimazole (MIC 6.25 μg/mL). Among these, compounds **6d$_1$**, **10a$_2$**, **10d$_2$**, **11d$_1$** **12a$_1$** and **12d** proved to be the most potent antifungal agents with MIC value 12.5 μg/mL and are half as potent as Clotrimazole. The analogs **4c**, **4d$_2$**, **5c**, **5d**, **6a$_2$**, **6c**, **6d$_2$**, **8a$_5$**, **9a$_2$**, **9a$_4$**, **10a$_6$**, **10c**, **11a$_2$**, **12a$_2$** and **12b** exhibited recognizable antifungal activity (MIC 25 μg/mL) that represented 25% of the standard's activity. It has been found that all of the tested compounds have low or no antifungal activity against *Aspergillus niger* (Table 3).

A close examination of the structures of the active compounds revealed that the antimicrobial profile of the thiobarbituric and barbituric compounds **4**–**12** seemed to be more favorable than their parent urea and thiourea derivatives **2** and **3** respectively, as evidenced by their IZ diameters and MIC values recorded in Table 3. However, it is clear from the observed data that the thiobarbituric acid derivatives **4** are more active than the corresponding barbituric acids **5**. The enhancement in the antimicrobial activity of TBA analogs when compared with BA derivatives suggests that electronic factors are more important for biological activity than geometrical factors. It was proposed that sulfur in C=S group is a good electron donor and hydrogen bond acceptor [3]. The second variable in the biological activity modulation is a trifluoroacetyl group in C-5 position of the thiobarbituric or barbituric nucleus and it has resulted in a remarkable improvement in the overall antimicrobial profile. Compound **6d$_2$** showed two folds increase in the activity against *S. aureus*, *B. subtilis* and *C. albicans* when compared with the parent **4d$_2$**, whereas the analog **6c** revealed a distinctive antimicrobial activity against all the tested organisms. It showed equipotent activity to Ampicillin against *S. aureus* (MIC 6.25 μg/mL), equipotent activity against *B. subtilis* and *P. aeruginosa* (MIC 12.5 μg/mL) and 50% of its activity against *E. coli* (MIC 12.5 μg/mL), together with an appreciable antifungal activity against *C. albicans* (MIC 25 μg/mL). On the other hand, cyclization of the thiobarbituric acid derivatives **4** to the pyrimido[4,5-d]pyrimidine derivatives **8** results in decreasing the reactivity of most of the tested compounds. Furthermore, derivatization of the parent barbituric acids **4** and **5** into the benzylidene functions, enhance the activity of compounds **9** and **10** toward all the tested microorganisms especially those with fluorine atom in their structures. Meanwhile cyclization of compounds **6** and **7** to the pyrazolo-pyrimidine derivatives **11** and **12** gave rise to moderately active compounds, and again the trifluoromethyl moiety play an essential role in increasing the activity toward most of the tested microorganisms.

2.2.2. Antidiabetic activity

Compounds **2a$_1$**, **2a$_2$**, **2c**, **3a$_1$**, **3a$_2$**, **4a$_1$**, **4c**, **5a$_1$**, **5a$_2$**, **6a$_1$**, **6c**, **7a$_1$**, **7c**, **8a$_1$**, **9a$_1$**, **9a$_2$**, **10a$_2$**, **11a$_1$**, **11a$_2$**, **12a$_1$**, **12d** were tested for hypoglycemic activity using alloxan-treated female albino mice.

Table 4
Antidiabetic activity of compounds **2**–**12**.

Compounds	Reduction in plasma glucose level, %	*P*
Phenformin	10	<0.01[a]
2a$_1$	4	0.05
2a$_2$	3	0.05
2c	5	0.05
3a$_1$	15	<0.01[a]
3a$_2$	14	<0.01[a]
4a$_1$	6	0.05
4c	7	0.01[a]
5a$_1$	4	0.05
5a$_2$	2	0.05
6a$_1$	8	<0.01[a]
6c	9	<0.01[a]
7a$_1$	5	0.05
7c	2.5	0.05
8a$_1$	<1	0.05
9a$_1$	5	0.05
9a$_2$	7	0.01[a]
10a$_2$	7.5	0.01[a]
11a$_1$	6	0.05
11a$_2$	7	0.01[a]
12a$_1$	3	0.05
12d	4	0.05

[a] Statistically significant.

From the data presented in Table 4, it is obvious that the 1,3-disubstituted urea derivatives (**3a$_1$** and **3a$_2$**) possess marked hypoglycemic activity. The potency of these compounds is more than that of phenformin. The corresponding thiourea derivatives (**2a$_1$**, **2a$_2$** and **2c**) however, have shown a marked reduction in the hypoglycemic activity suggesting a negative influence of electronic effects of sulphur. On the other hand although the activity of the thioureas **2** is low, their cyclic thio-analogs **4** have shown an improvement in the activity. Here geometric factor becomes an important variable in enhancing the biological effects. Introduction of trifluoroacetyl moiety at position 5 of the thiobarbituric acid ring enhances the hypoglycemic activity of the thiobarbituric acid derivatives **6**.

3. Conclusions

The synthesized benzenesulfonamide derivatives of barbituric and thiobarbituric acids have shown promising antibacterial, antifungal and antidiabetic activities. The antimicrobial profile of thiobarbiturates was found to be more promising than the corresponding barbiturates. The introduction of trifluoroacetyl group at C-5 has certainly improved the overall antimicrobial activity of barbituric as well as thiobarbituric acids. Almost all compounds **4**–**12** have shown very good antimicrobial activity. However, some structural prototypes viz., **6c**, **6d$_2$** and **10c** were found to be equipotent with Ampicillin. On the other hand, the hypoglycemic activity was reduced in thiobarbiturates when compared from the corresponding barbituric acid derivatives. Compounds **3a$_1$** and **3a$_2$** have been selected for further structure optimization.

4. Experimental

4.1. Chemicals and methods

Melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus and were uncorrected. The infrared (IR) spectra were recorded on Perkin-Elmer 297 infrared spectrophotometer using the plate technique. The ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 as a solvent on Bruker DPX-400-FT spectrometer using tetramethylsilane as the internal standard. Elemental analyses were performed at the Microanalytical Unit, Faculty of Science, Cairo University, Cairo, Egypt. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, E. Merck) and the spots were detected by exposure to UV lamp at λ 254. Biological testing was performed in the Faculty of Medicine University of Alexandria, Egypt. Reagents were of analytical grade and were used without further purification.

4.1.1. *N,N′-disubstituted thioureas (**2a–d**)*

A mixture of appropriate sulfonamide **1** (10 mmol) in DMF (20 mL) and the appropriate isothiocyanate (10 mmol) in dioxin (20 mL) was heated on a water bath for 1 h, cooled and then poured onto ice cooled water. The pale yellow solid thus obtained was filtered and recrystallized from ethanol as needles.

4.1.2. *N,N′-disubstituted ureas (**3a–d**)*

A mixture of appropriate sulfonamide **1** (10 mmol) in DMF (20 mL) and the appropriate isocyanate (10 mmol) in dioxin (20 mL) was heated on a water bath for 1 h, cooled then poured onto ice cooled water. The pale yellow solid thus obtained was filtered off and recrystallized from ethanol as needles.

4.1.3. 1,3-Disubstituted-2,3-dihydro-2-thioxo-4,6(1H,5H)pyrimidine-diones (**4a–d**)

A mixture of the appropriate thiourea derivative **2** (10 mmol) and malonic acid (15 mmol) in acetyl chloride (10 mL) was heated on an oil bath for 10 h at 40 °C. The reaction mixture was then cooled and poured onto crushed ice and the resulting solid was filtered, washed with water and recrystallized from ethanol as needles.

4.1.4. 1,3-Disubstituted-pyrimidine-2,4,6-triones (**5a–d**)

A mixture of the appropriate urea derivative **3** (10 mmol) and malonic acid (15 mmol) in acetyl chloride (10 mL) was heated on an oil bath for 12 h at 50 °C. The reaction mixture was then cooled and poured onto crushed ice and the precipitated solid was filtered off and recrystallized from ethanol as needles.

4.1.5. 1,3-Disubstituted-5-trifluoroacetyl-2,3-dihydro-2-thioxo-4,6(1H,5H)pyrimidine-diones (**6a–d**)

A solution of the appropriate thiopyrimidine **4** (10 mmol) in THF (25 mL) was refluxed with trifluoroacetic anhydride (10 mmol) for 2 h. The reaction mixture was then cooled and poured into water; the precipitated trifluoroacetyl derivative was recrystallized from ethanol as needles.

4.1.6. 1,3-Disubstituted-5-trifluoroacetylpyrimidine-2,4,6-triones (**7a–d**)

A solution of the appropriate pyrimidine **5** (10 mmol) in THF (25 mL) was refluxed with trifluoroacetic anhydride (10 mmol) for 2 h. The reaction mixture was then cooled and poured into water; the precipitated trifluoroacetyl derivative was recrystallized from ethanol as needles.

4.1.7. 1,3,5-Trisubistituted-2,7-dithioxo-octahydropyrimido[4,5-d]pyrimidin-4-ones (**8**)

Aromatic aldehydes (10 mmol), substituted thiobarbituric acid (10 mmol) and thiourea (10 mmol) were dissolved in ethanol (20 mL) and the mixture refluxed on a water bath in the presence of a catalytic amount of concentrated HCl. The progress of the reaction was monitored by TLC. After completion of the reaction, the concentrated reaction mixture was cooled and poured onto ice-cold water. The solid that separated was filtered, dried, and recrystallized from absolute alcohol.

4.1.8. 5-Arylidine-1,3-disubistituted-2-thioxo-dihydropyrimidine-4,6-diones (**9**)

A solution of the appropriate thiobarbituric acid **4** (10 mmol) in absolute ethanol (25 mL) containing a catalytic amount of piperidine (0.5 mL) was refluxed with the appropriate aromatic aldehyde (10 mmol) in the presence of few drops of glacial acetic acid for 4 h. The reaction mixture was then cooled and poured onto water; the precipitated arylidine derivative was recrystallized from ethanol as needles.

4.1.9. 5-Arylidine-1,3-disubistituted pyrimidin-2,4,6-triones (**10**)

A solution of the appropriate barbituric acid **5** (10 mmol) in ethanol (25 mL) was refluxed with the appropriate aromatic aldehyde (10 mmol) in the presence of few drops of glacial acetic acid for 4 h. The reaction mixture was then cooled and poured onto water. The solid which separated out was filtered off, dried, and recrystallized from absolute alcohol.

4.1.10. 5,7-Disubistituted-6-thioxo-3-(trifluoromethyl)-6,7-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (**11**)

A solution of the appropriate thiobarbituric acid **5** (10 mmol) in DMF (20 mL) was refluxed with hydrazine hydrate (12 mmol) for 4 h. The reaction mixture was then cooled and poured onto ice water; the precipitated trifluoromethyl-pyrazolo-pyrimidine derivative was recrystallized from ethanol as needles.

4.1.11. 5,7-Disubistituted-3-(trifluoromethyl)-1H-pyrazolo[3,4-d]pyrimidine-4,6(5H,7H)-diones (**12**)

A solution of the appropriate trifluoroacetyl-pyrimidine **6** (10 mmol) in DMF (20 mL) was refluxed with hydrazine hydrate (12 mmol) for 4 h. The reaction mixture was then cooled and poured onto ice water. The solid that separated was filtered off, dried, and recrystallized from absolute alcohol as needles.

4.2. Biological evaluation

4.2.1. In vitro antibacterial and antifungal activities

Standard sterilized filter paper discs (5 mm diameter) impregnated with a solution of the test compound in DMSO (1 mg/mL) was placed on an agar plate seeded with the appropriate test organism in triplicates. The utilized test organisms were: *S. aureus* (ATCC 6538) and *B. subtilis* (NRRL B-14819) as examples of Gram positive bacteria and *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) as examples of Gram negative bacteria. They were also evaluated for their in vitro antifungal potential against *C. albicans* (ATCC 10231) and *A. niger* (recultured) fungal strains were utilized as representatives for fungi. Ampicillin trihydrate and Clotrimazole were used as standard antibacterial and antifungal agents, respectively. DMSO alone was used as control at the same above-mentioned concentration. The plates were incubated at 37° C for 24 h for bacteria and for 7 days for fungi. Compounds that showed significant growth inhibition zones ($\geq$14 mm) using the two-fold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

4.2.2. Minimal inhibitory concentration (MIC) measurement

The micro-dilution susceptibility test in Müller-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively [16]. Stock solutions of the tested compounds, Ampicillin trihydrate and Clotrimazole were prepared in DMSO at concentration of 800 μg/mL followed by two-fold dilution at concentrations of 200, 100, 50, 25, and 12.5 μg/mL. The microorganism suspensions at 10^6 CFU/mL (colony forming unit/mL) concentrations were inoculated to the corresponding wells. Plates were incubated at 36 °C for 24–48 h and the minimal inhibitory concentrations (MIC) were determined. Control experiments were also done.

4.2.3. Antidiabetic activity

Twenty one compounds were tested for hypoglycemic activity using alloxan-treated female albino mice weighing 20 g. Alloxan 100 mg/kg was injected into the tail vein in a 10 mg/mL saline solution. Three days later the mice were given the test compounds orally in suspension in 1% carboxymethylcellulose solution at the rate of 0.2 mmol/kg of the body weight. Each day a group of four mice was used as a control group and one group of five mice was given the standard 100 mg of phenformin/kg. Up to six groups of four mice received the test compounds. Blood samples were collected into 0.04% NaF solution at 0, 1 and 3 h.

Glucose was determined by the micro-colorimetric copper reduction technique of Haslewood and Strookman [17]. Results are expressed as a percentage reduction of the plasma glucose levels compared with the control value. Statistical significance was assessed by a Student's *t*-test. Statistical significance was accepted where the calculated *t*-value exceeded the tabulated *t*-value at the $p = 0.05$ level.

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