# **Full Paper**

# Synthesis and Biological Evaluation of Novel Pyrazoles and Pyrazolo[3,4-*d*]pyrimidines Incorporating a Benzenesulfonamide Moiety

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Synthesis and biological evaluation of novel pyrazoles and pyrazolo[3,4-*d*]pyrimidines are reported. Fourteen compounds were selected by the NCI and tested for their preliminary *in-vitro* anticancer activity, whereas all the synthesized compounds were evaluated for their *in-vitro* antimicrobial activity. Compound **12a** was proven to possess the highest anticancer activity with a broad spectrum profile. It showed particular effectiveness towards leukemia HL-60 (TB), K-562, non-small cell lung cancer NCI-H23, and colon cancer HT 29, KM 12 cell lines (GI<sub>50</sub> = 6.59, 4.44, 1.37, 3.33, and 9.63 µM, respectively). Out of the synthesized compounds, thirteen derivatives were found to display pronounced antimicrobial activity especially against *P. aeruginosa*. Compounds **2c**, **5b**, **10**, **11b**, **17b**, **18b**, and **19** were proven to be the most active with a broad spectrum of activity. Compound **19** was found to be equipotent to ampicillin against *B. subtilis*, whereas compounds **11b** and **19** were four times superior to ampicillin against *P. aeruginosa*, while compounds **2c**, **10**, and **11b** were nearly equipotent to ampicillin against *E. coli*. On the other hand, compounds **2c**, **5b**, **10**, **11a**, **17b**, and **18b** exerted nearly half the activity of clotrimazole against *C. albicans*.

Keywords: Antimicrobial activity / Anticancer activity / Benzenesulfonamide / Pyrazoles / Pyrazolo[3,4-d]pyrimidines

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#### Introduction

In the last few years, much attention has been focused on pyrazoles due to their diverse biological activities. Pyrazole-containing structures have been found among naturally occurring compounds such as the antibiotic pyrazofurin which has been documented to possess potent anticancer, antimicrobial, and broad-spectrum antiviral activity against many RNA and DNA viruses [1]. Recently, extensive studies have been devoted to N-aryl pyrazole derivatives [2–5] after the discovery of the antitumor and anti-angiogenic properties [6, 7] of celecoxib and SC-236 [8] (Fig. 1). Celecoxib has been proved to be effective for the treatment of familial adenomatous polyposis and ongoing clinical trials are currently assessing its potential therapeutic role in both prevention and treatment of a diverse range of human cancers [9]. Moreover, pyrazole derivatives acting as selective inhibitors of bacterial DNA gyrase have also been reported [10-12]. In addition, fused pyrazoles such as pyrazolopyrimidines and related fused heterocycles have displayed an impressive array of pharmacological activities. They have been identified as adenosine antagonists, tyrosine kinase, and Src-kinase inhibitors [13-19]. Purine derivatives such as olomoucine and roscovitine (Fig. 1); structurally related to pyrazolo[3,4-d]pyrimidine compounds were found to exhibit moderate inhibitory activity but good selectivity for a panel of cyclin-dependent kinases (CDK) [20] which play a key role during cell division. Recently, it was reported

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Abbreviations: mean-graph midpoint values (MG-MID); minimum bacterial concentration (MBC); minimal inhibitory concentration (MIC)



**Figure 1.** The structure of some biologically active pyrazoles and pyrazolo[3,4*d*]pyrimidines.

that a pyrazolo[3,4-*d*]pyrimidin-4-one subunit is a significant antimicrobial activity enhancing group which inhibits DNA polymerase III [21].

On the other hand, antibacterial sulfonamides continue to play an important role in chemotherapy either alone or in combination with other drugs. Besides, classical human carbonic anhydrase inhibitors characterized by the presence of aromatic or heteroaromatic sulfonamide scaffolds have gained much interest as possible antitumor agents [22–24] especially after the discovery of overexpression of human carbonic anhydrase IX in many cancer tissues when compared to normal cells [25].

The above-mentioned findings, coupled with our interest in the chemistry of pyrazoles [26–28] envisioned our approach towards the investigation of novel pyrazoles and pyrazolo[3,4-*d*]pyrimidines incorporating a benzenesulfonamide moiety as anticancer and antimicrobial agents. These compounds were designed to comprise as a general feature the benzenesulfonamide moiety attached to N-1 of the biodynamic pyrazole ring. Such structural assembly was supposed to encourage the biological potential of this class of compounds. The aim of the present study is the discovery of novel structure leads that might be of use in designing new compounds endowed with both anticancer and antimicrobial properties.

To ameliorate the pharmacological profile of the pyrazole derivatives it became worthwhile to introduce the amidic pharmacophores which are believed to be responsible for the biological significance of some relevant natural and synthetic chemotherapeutic agents [29, 30]. Moreover, Schiff bases with their effective contribution as potential chemotherapeutic agents [31, 32] were not far of our attention. Regarding the pyrazolo [3,4-d] pyrimidine derivatives, it was planned to introduce variable substituents in position 4 of the pyrazolopyrimidine core structure. Such substituents were selected to confer different electronic and lipophilic characteristics to the molecules. Moreover, owing to the well documented therapeutic potential associated with triazoles [33, 34], we deemed it interesting to prepare compounds incorporating the triazole ring fused with the pyrazolopyrimidine nucleus in order to explore the influence of such structural variation on the anticipated biological activities.

## **Results and discussion**

#### Chemistry

Synthesis of the intermediate and target compounds was performed according to the reactions outlined in

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Schemes 1, 2, and 3. The amino ester **2a** was prepared as previously described [35]. Synthesis of carboxamides 2b, c was achieved by refluxing a mixture of the selected N-substituted-2-cyano-3-ethoxyprop-2-enamides 1b, c and 4-hydrazinobenzenesulfonamide hydrochloride in ethanol containing anhydrous sodium acetate. Heating the amino ester 2a in hydrazine hydrate 80%, gave rise to the target acid hydrazide 3, which, upon treatment with acetyl or benzoyl chloride in dry pyridine, resulted in the corresponding acyl derivatives 4a, b. The arylidene derivatives **5a**, **b** were prepared following previously reported reaction conditions [36]. Refluxing 5a, b with thioglycolic acid in dry dioxane afforded the respective thiazolidinones 6a, b. Synthesis of oxadiazole-2-thiol 7 was achieved following the reaction conditions adopted for the preparation of related compounds [37]. Reacting the acid hydrazide 3 with an equimolar amount of acetyl acetone in refluxing ethanol, yielded the corresponding 3,5dimethylpyrazole 8. The key intermediate thione 10 was prepared from compound 9 [35] utilizing the same procedure for the preparation of allied compounds [38]. The Salkyl analogs 11a-d were prepared according to the procedure reported by Tromp et al. [39]. Fusion of the methylthio analog 11a with the selected amine at 160-170°C afforded the respective substituted amino derivatives 12a-d. On the other hand, refluxing 11a with 98% hydrazine hydrate in ethanol furnished the corresponding hydrazine derivative 13, which was employed as key intermediate in Scheme 3. Thus, heating 13 with ethyl chloroformate in dry pyridine gave rise to the ethyl carboxylate derivative 14. Refluxing the hydrazine derivative 13 in glacial acetic acid yielded the target pyrazolotriazolopyrimidine 15. Synthesis of arylidene derivatives 16a, b was accomplished adopting the same procedure for preparation of compounds 5a, b. Cyclocondensation of 13 with phenacyl or 4-chlorophenacyl cyanide gave rise to the requisite 5-aminopyrazolyl derivatives 17a, b. Synthesis of N-substituted thiocarbamoylhydrazino derivatives **18a**, **b** was accomplished by stirring a mixture of 13 and the proper aryl isothiocyanate in DMF at room temperature. The proposed tricyclic analog 19 was prepared by refluxing 13 with carbon disulfide in dry pyridine. It should be noted that compound 19 was unexpectedly separated on refluxing the hydrazine derivative 13 with aryl isothiocyanates in dry dioxane.

#### Preliminary in-vitro anticancer screening

Out of the newly synthesized compounds, 14 derivatives namely: **2a**, **8**, **10**, **11a**–**d**, **12a**–**d**, **16a**, **18a**, and **19** were selected by the National Cancer Institute (NCI) *in-vitro* disease-oriented human cells screening panel assay to be evaluated for their *in-vitro* antitumor activity. An effective

tive one-dose assay has been added to the NCI-60 cell screen in order to increase compound throughput and reduce data-turnaround time to suppliers while maintaining efficient identification of active compounds [40, 41]. All compounds submitted to the NCI-60 cell screen are now tested initially at a single high dose (10 µM) in the full NCI-60 cell panel including leukemia, non-small cell lung, colon, CNS melanoma, ovarian, renal, prostate, and breast cancer cell lines. Only compounds which satisfy pre-determined threshold inhibition criteria would proceed to the five-dose screen. The threshold inhibition criteria for proceeding to the fivedose screen was designed to efficiently capture compounds with anti-proliferative activity, and it is based on careful analysis of historical Development Therapeutic Program (DTP) screening data. Data are reported as a mean graph of the percent growth of treated cells, and presented as percentage growth inhibition (GI%) caused by the test compounds (Table 1). Moreover, three response parameters (GI<sub>50</sub>, TGI, and LC<sub>50</sub>) were calculated for each cell line for compound 12a (Table 2). The  $GI_{50}$ value corresponds to the compound concentration causing 50% decrease in net cell growth. The TGI value is the compound concentration resulting in total growth inhibition and the LC<sub>50</sub> value is the compound concentration causing a net 50% loss of initial cells at the end of the incubation period (48 h). Subpanel and fullpanel mean-graph midpoint values (MG-MID) for certain agents are the average of individual real and default GI<sub>50</sub>, TGI, or LC<sub>50</sub> values of all cell lines in subpanel and fullpanel, respectively [42].

A deep insight into the percentage growth inhibition of the test compounds (Table 1) revealed that some of the tested subpanel tumor cell lines exhibited pronounced sensitivity profiles against most of the tested compounds. Among these, the non-small cell lung cancer HOP-92 cell line exhibited a variable degree of sensitivity towards nine out of the 14 compounds selected with particular sensitivity towards compounds 12a and 18a (GI% = 82.2 and 78.9, respectively). Furthermore, the CNS cancer SF-295 cell line demonstrated remarkable sensitivity towards ten out of the 14 compounds selected, with particular sensitivity toward compounds 12a, 16a, and 19 (GI% = 70.5, 70.2, and 87.1%, respectively). In addition, growth of the renal cancer CAKI-1, RXF 393, and UO-31 cell lines was variably affected by ten out of the tested compounds. Special high activity against CAKI-1 was shown by compounds **11b** and **11c** (GI% = 73.7 and 92.2%, respectively); whereas remarkable activity against the RXF 393 cell line was displayed by 12a, 12d, 16a, and 19 (GI% = 89.1, 86.6, 118.8 and 82.2%, respectively). It should be noted here that compound 16a showed lethal effect

Table 1	. In viti	ro percentag	e growth	inhibition	(GI%)	caused	by the	test	compounds	against	some	selected	tumor	cell lines	at the
single de	ose ass	say <sup>a)</sup> .	-				-			-					

Compound	NSC No.	Panel	Subpanel tumor cell lines (% growth inhibitory activity)
2a	747468	Renal Cancer	UO-31 (35.2)
		CNS Cancer	SNB-75 (21.9)
8	747472	CNS Cancer	SNB-75 (21.9)
10	742615	Non-Small Cell Lung Cancer	HOP = 92 (45.9)
		Ovarian Cancer	IGROV1(21.2)
		Leukemia Bonal Cancor	HL-6U(1B)(21.3)
		Prostate Cancer	CARF1 (32.0), RAF 393 (30.8) $DC_3 (12.5)$
		CNS Cancer	SE-295 (36)
11a	742616	Non-Small Cell Lung Cancer	HOP-92(32,2)
114	/ 12010	Leukemia	HI-60 (TB)(30.3), K-562(2.3.4), MOLT-4(2.0)
		Renal Cancer	CAKI-1 (61.2), RXF 393 (68)
		CNS Cancer	SF-295 (41.2)
11b	742617	Non-Small Cell Lung Cancer	A549/ATCC (19.2), HOP-62(33), HOP-92(57).
		Colon Cancer	CT 116 (17.3)
		Leukemia	K-562 (25.24), MOLT-4 (49.7)
		Renal Cancer	CAKI-1 (73.7)
		Melanoma	M14 (33), MALME-3M (17.2), SK-MEL-5(20.5), UACC-257(43.4).
		Prostate Cancer	DU-145 (21.9)
		CNS Cancer	SF-295 (35.5).
11c	742618	Non-Small Cell Lung Cancer	HOP – 92 (49)
		Colon Cancer	HCT-116 (29.4), SW-620 (26.5)
		Breast Cancer	MCF 7 (16.9), MDA-MB-435 (23.8).
		Ovarian Cancer	VVCAK-3 (15.6), $VVCAK-8$ (16.5)
		Leukemia Bonol Concor	HL-60 (1B)(27.6), K-562(36.8), $SK(42.9)$
		Melanoma	CARI-1 (92.2), KAF 393 (05.8) MAIME-3M(30.9) SK-MEL-5(20.9) IIACC-257(34.5)
		CNS Cancer	SE-295 (45 5)
11d	742619	Non-Small Cell Lung Cancer	HOP-62(18) HOP-92(42.2)
114	, 12015	Renal Cancer	RXF 393 (63.7)
		Melanoma	UACC-257 (20.5)
		CNS Cancer	SF-295 (63), SNB-19 (16.9)
12a	742621	Non-Small Cell Lung Cancer	A549/ATCC (24), HOP-92(82.2), NCI-H322M (28.1).
		Colon Cancer	HCT-15 (27.6), HT29 (58.6), KM12 (24.5).
		Ovarian Cancer	IGROV1 (43.3)
		Leukemia	HL-60 (TB) (47.8), K-562 (61.8), MOLT-4(31.2), RPMI-8226 (47.3).
		Renal Cancer	CAKI-1 (62), RXF 393 (89.1), TK-10(29.2), UO-31(84.7).
		Melanoma	LOXIMVI(29.8), M14(30.4), SK-MEL-5(20.3), UACC-257(18.6)
		Prostate Cancer	PC-3 (20.5).
10h	747471	Non Small Coll Lung Cancor	3F-295(70.5), 0251(24.9).
120	/4/4/1	Colon Cancer	HOT = 52(23.4) $HCT_{116}(10.0) HCT_{15}(17.0) HT 20(35.5)$
		Breast Cancer	MDAMB-468 (25.4)
		Leukemia	CCRF-CEM (46.7), HI-60(TB)(35.7), K-562(49.5), RPMI-8226(28.9).
		Renal Cancer	RXF 393 (22.1), SN 12C (19.6), UO-31 (27).
		Melanoma	M14 (16.6).
		CNS Cancer	SNB-75 (17.4), U251 (18.6).
12c	742622	Non-Small Cell Lung Cancer	NCI-H322M (19.4).
		Breast Cancer	MDA – MB – 231/ATCC (17.2) , NCI / ADR-RES (30.1) ,T-47D (18.3)
		Ovarian Cancer	IGROV1 (40.4).
		Leukemia	K-562 (37.4), SR (57.5).
		Renal Cancer	CAKI-1 (29.7), UO-31 (36.1).
		Melanoma	LOX IMVI (22.6), SK-MEL-5 (24.1).
12d	742623	Non-Small Cell Lung Cancer	A549/ATCC (17), HOP-92 (52.5).
		Colon Cancer	H129 (18.1), KM12 (16.4).
		Breast Cancer	1-4/ <i>D</i> (19.6).
		Uvarian Cancer	$H_{L}(0,TD)(20,2)$ K = (2, (4 = 5) MOLT 4 (27,4) SD (26, 8)
		Leukenna Repal Cancer	ПЬ-0U (1B) (29.3), N-302 (43.3), MULI-4 (27.4), SK (30.8). САКЬТ (60.4), RYE 303 (86.6), ЦО 21/00.1)
		Melanoma	$I \cap XI M VI (19.2) IIA (C-257/20.4)$
		CNS Cancer	SF-295 (51.1).

#### Table 1. Continued.

Compound	NSC No.	Panel	Subpanel tumor cell lines (% growth inhibitory activity)
16a	742624	Non-Small Cell Lung Cancer	A549/ATCC (48), NCI-H23 (19.4), NCI-H460 (33.6)
		Colon Cancer	HCC-2998(25.3), HCT-116(16.8), HT 29 (19.1)
		Ovarian Cancer	IGROV1 (23.5), OVCAR-4 (45.7), OVCAR-8 (44.5)
		Leukemia	HL-60(TB) (16.9), K-562 (16.9), MOLT-4 (28.6), SR (49.2).
		Renal Cancer	CAKI-1 (46.6), RXF 393 (118.8), UO-31 (31.2)
		Melanoma	SK-MEL-2 (29.5), UACC-257(37.6).
		Prostate Cancer	DU-145(26.9), PC-3 (19.4).
		CNS Cancer	SF-268(23.1), SF-295(70.2), SF-539(23.8), SNB-19 (19.6).
18a	742625	Non-Small Cell Lung Cancer	HOP-62 (24.4), HOP-92 (78.9).
		Renal Cancer	CAKI-1 (66).
		Melanoma	M14 (20.3).
		CNS Cancer	SF-295 (50.4).
19	742627	Non-Small Cell Lung Cancer	HOP-92 (33.5).
		Ovarian Cancer	IGROV1 (32)
		Renal Cancer	RXF 393 (82.2).
		Melanoma	UACC-257 (20.4).
		CNS Cancer	SF-295 (87.1).

 $^{a)}$  The data obtained from NCI *in-vitro* disease-oriented human tumor cell screen at 10  $\mu$ M concentration.

Panel	(Subpanel cell lines) (Cytotoxicity $GI_{50} \mu M$ )
Leukemia	CCRF-CEM (17.70), HL-60 (TB) (6.59), K-562 (4.44), MOLT-4 (14.50), RPMI-8226 (15.30), SR (29.60)
Lung Cancer	A549 / ATCC (14.30), EKVX (41.40), HOP-62 (16.70), HOP-92 (23.60), NCI-H226 (46.40), NCI-H23 (1.37), NCI-H322M (31.50), NCI-H460 (14.90), NCI-H522 (51.10)
Colon Cancer	COLO205 (21.10), HCC-2998 (16.60), HCT-116 (27.40), HCT-15 (19.10), HT-29 (3.33), KM 12 (9.63), SW-620 (30.30)
CNS Cancer	SF-268 (43.10), SF-295 (16.90), SF-539 (36.20), SNB-75 (33.50), U251 (26.90)
Melanoma	LOX IMVI (14.90), MALME-3M (33.00), M14 (32.80), SK-MEL-2 (44.30), SK-MEL-28 (31.60), SK-MEL-5 (24.40), UACC-257 (39.40), UACC-62 (42.80)
Ovarian Cancer	OVCAR-3 (38.20), OVCAR-4 (32.10), OVCAR-5 (34.90), OVCAR-8 (18.30)
Renal Cancer	786-O (18.30), A498 (24.30), ACHN (38.50), CAKI-1 (23.70), RXF 393 (29.60), TK-10 (19.10), UO-31 (23.90)
Prostate Cancer	PC-3 (35.10), DU-145 (35.60)
Breast Cancer	MCF7 (49.20), NCI / ADR-RES (19.50), MDA- MB- 231 / ATCC (15.0), HS 578T (33.80), MDA-MB-435 (46.10), BT-549 (30.30), T-47D (24.20)

Table 2. Growth inhibitory a	action (GI <sub>50</sub> ) of	f some selected <i>in-vitro</i> tumor cell lines	(μM) <sup>a)</sup> for c	compound 12a	(NCS 742621).
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<sup>a)</sup> Data obtained from NCI *in-vitro* disease-oriented human cell screen.

towards the RXF 393 cell line with 18.8%. However, growth of the UO-31 cell line was affected by three compounds, namely: **2a**, **12a**, and **12d** (GI% = 35.2, 84.7, and 99.1, respectively). In addition, the colon cancer HT 29 was only reasonably inhibited by compound **12a** (GI% = 58.6), whereas growth of ovarian cancer OVCAR-4 and OVCAR-8 was inhibited by compound **16a** (GI% = 45.7 and 44.5, respectively). More importantly, appreciable activity against leukemia SR cell line was manifested by compounds **11c**, **12c**, and **16a** (GI% = 42.9, 57.5, and 49.2, respectively), while moderate activity against leukemia K 562 cell line was shown by compounds **12a**, **b** and **d** (GI% = 61.8, 49.5, and 45.5, respectively). The rest of the tested tumor cell lines showed weak activity or no sensitivity towards the tested compounds (GI% = 0–20). Overall, it

can be concluded that the pyrazolopyrimidine derivatives showed considerable activity against the tested tumor cell lines, whereas the pyrazole derivatives showed very weak or no activity, and only compound **12a** fulfilled the requirements of selection for five-dose assay.

Further interpretation of the five-dose screening data for compound **12a** (Table 2) revealed that this compound was proven to be the most active member in this study with a broad spectrum of activity against most of the tested subpanel tumor cell lines with particular effectiveness against leukemia K-562, lung cancer NCI-H23, and colon cancer HT 29 (GI<sub>50</sub> = 4.44, 1.37, 3.33  $\mu$ M and TGI = 37.7, 23.8, and 15.7  $\mu$ M, respectively). The compound also showed significant activity against leukemia HL-60 (TB) and colon cancer KM 12 (GI<sub>50</sub> = 6.59 and 9.63  $\mu$ M, respec-

MG-MID <sup>a)</sup>	Subpanel tumor cell lines <sup>b)</sup> $GI_{50}$ MG-MID ( $\mu$ M) (SI) <sup>c)</sup>								
	I	II	III	IV	V	VI	VII	VIII	IX
28.93	14.7 (1.97)	26.8 (1.08)	18.2 (1.59)	31.3 (0.92)	32.9 (0.88)	44.7 (0.65)	25.3 (1.14)	35.3 (0.82)	31.2 (0.93)

Table 3. The MG-MID (GI<sub>50,  $\mu$ M) and the selectivity ratio of compound **12a** (NSC 742621).</sub>

 $^{\mathrm{a})}~GI_{50}\text{:}$  Full panel mean-graph midpoint ( $\mu M\text{)}.$ 

<sup>b)</sup> I: leukemia; II: non-small cell lung cancer; III: colon cancer; IV: CNS cancer; V: melanoma; VI: ovarian cancer; VII: renal cancer; VIII: prostate cancer; IX: breast cancer.

<sup>c)</sup> SI: selectivity index.

tively). In addition, it displayed moderate activity against leukemia CCRF-CEM, MOLT-4, RPMI-8226, lung cancer A 549/ATCC, HOP-62, NCI-H 460, colon cancer HCC-2998, HCT-15, CNS cancer SF 295, melanoma LOX IMVI, ovarian cancer OVCAR-8, renal cancer 786-0, TK-10 and Breast NCI/ADR-RES, MDA-MB-231/ATCC cell lines (GI<sub>50</sub> range from 14.3 to 19.5  $\mu$ M). The LC<sub>50</sub> (cytotoxicity values) were >100  $\mu$ M for all tested cell lines.

The ratio obtained by dividing the compound fullpanel MG-MID ( $\mu$ M) by its individual subpanel MG-MID ( $\mu$ M) is considered as a measure of compound selectivity (Table 3). Ratios between 3 and 6 refer to moderate selectivity, ratios >6 indicate high selectivity toward the corresponding cell line, while compounds meeting neither of these criteria are rated non-selective [40]. Accordingly, compound **12a** proved to be non-selective.

#### Antimicrobial screening

All the newly synthesized compounds were evaluated for their in-vitro antibacterial activity against Staphylococcus aureus and Bacillus subtilis as Gram-positive bacteria, Escherichia coli and Pseudomonas aeruginosa as Gram-negative bacteria. They were also evaluated for their in-vitro antifungal potential against Candida albicans. Their inhibition zones using the cup-diffusion technique [43] were measured and further evaluation was carried out for compounds showing reasonable inhibition zones (>13 mm) to determine their minimal inhibitory concentration (MIC) and minimum bacterial concentration (MBC) using the twofold serial dilution method [44]. Ampicillin was used as standard antibacterial while clotrimazole was used as antifungal reference. Dimethylsulfoxide (DMSO) was used as blank and showed no antimicrobial activity.

As revealed from Tables 4 and 5, thirteen compounds displayed promising inhibitory effects on the growth of the tested Gram-positive and Gram-negative microorganisms with special high activity against *P. aeruginosa*. Moreover, they exhibited variable degrees of antifungal activity against *C. albicans*. Concerning the antibacterial

Table 4.	The	inhibition	zones	(IZ)	in mm	diameter	of	the	most
active co	mpοι	unds.							

Compou	nd S. aureus	B. subtilis	P. aeruginosa	E. coli	C. albicans
2c	18	15	14	12	11
5b	14	17	16	15	15
9	18	18	15	15	14
10	13	11	15	13	16
11a	12	13	14	14	13
11b	12	13	13	14	14
11c	13	16	18	16	16
12b	13	18	18	17	15
12c	14	18	18	16	15
13	20	21	19	18	17
17b	18	18	13	18	14
18b	14	17	16	15	15
19	17	18	18	14	14

potency of the active compounds against S. aureus, compounds **11c** and **17b** (MIC =  $12.5 \,\mu g/mL$ ) displayed nearly half the activity of ampicillin, whereas the remaining compounds showed mild to weak activity. With regard to the activity against B. subtilis, only compound 19 was found to be equipotent to ampicillin while compounds 5b, 11b, 12b, 12c, 13, 17b, and 18b were four times less active than ampicillin against the same organism. Interestingly, P. aeruginosa was proven to be the most sensitive microorganism to most of the tested compounds. Compounds **11b** and **19** (MIC =  $12.5 \,\mu g/mL$ ) were four times more active than ampicillin, whereas compounds 5b, 12c, and 18b exerted the same level of activity as ampicillin, while compounds 9, 11a, 11c, 12b, 13, and 17b showed half the activity of ampicillin. Furthermore, compounds 2c, 10, and 11b (MIC =  $12.5 \,\mu g/mL$ ) were nearly equivalent to ampicillin against E. coli, meanwhile the remaining compounds showed moderate to mild activity. On the other hand, investigation of the antifungal activity of the tested compounds revealed that six analogs, namely 2c, 5b, 10, 11a, 17b, and 18b were able to produce significant growth inhibition (MIC values = 12.5 µg/mL).

Compound	2	5. aureus	В	3. subtilis	Р. с	ieruginosa		E. coli	С.	albicans
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
2c	50	50	100	100	200	200	12.5	12.5	12.5	25
5b	100	100	50	50	50	50	100	100	12.5	25
9	100	200	100	100	100	100	50	50	50	50
10	50	50	100	100	200	200	12.5	50	12.5	50
11a	50	50	100	100	100	200	50	50	12.5	25
11b	50	50	50	50	12.5	25	12.5	12.5	50	50
11c	12.5	25	100	100	100	200	100	200	100	100
12b	200	200	50	50	100	100	100	100	50	50
12c	50	50	50	50	50	50	100	100	100	100
13	100	200	50	50	100	100	50	50	100	200
17b	12.5	12.5	50	50	100	100	50	50	12.5	12.5
18b	100	100	50	50	50	50	100	100	12.5	25
19	50	50	12.5	12.5	12.5	25	50	50	100	100
Ampicillin	5	-	12.5	-	50	-	10	-	-	-
Clotrimazole	-	-	-	-	-	-	-	-	5	-

Table 5. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the most active compounds in  $\mu$ g/mL.

According to the MIC and MBC limits derived from the latest National Committee on Clinical Laboratory Standards (NCCLS), it can be determined whether the test compound is bactericidal or bacteriostatic to the test organism [42]. Accordingly, and as revealed from Table 5, compound **17b** was bactericidal against *S. aureus* while compound **19** was bactericidal against *B. subtilis*. Compounds **5b**, **12c**, and **18b** were also bactericidal against *P. aeruginosa*. In addition, compounds **2c** and **11b** were bactericidal against *E. coli*, while compound **17b** was fungicidal against *C. albicans*. The remaining compounds were bacteriostatic against the test organisms.

The activity of the test compounds could be correlated to the structural variations and modification. Within the pyrazole series (Scheme 1), the nature of the substituent at position 4 seems to manipulate the antimicrobial activity. Although the phenyl pyrazolecarboxamide derivative 2b was totally inactive against all the tested organisms, the 4-chlorophenyl analog 2c displayed substantial antimicrobial activity against P. aeruginosa, E. coli, and C. albicans (MIC = 200, 12.5, 12.5 µg/mL, respectively), however, it showed weak antimicrobial activity against the remaining test organisms. The prototype 3 did not show any antimicrobial activity against the tested organisms. Acetylation, benzoylation, or condensation to the corresponding oxadiazolyl and 3,5-dimethylpyrazolyl derivatives (4a, b, 7, 8) did not result in any change in the microbiological profile. On the other hand, condensation of 3 with aromatic aldehydes afforded one active derivative which is the 4-chloro analog 5b that exhibited significant activity against B. subtilis, P. aeruginosa, and C. albicans (MIC = 50, 50, 12.5  $\mu$ g/mL). However, cyclization of **5b** to the thiazolidinyl counterpart **6b** completely abolished the antimicrobial activity.

Regarding the pyrazolo[3,4-d]pyrimidine derivatives (Schemes 2, 3), the results revealed that the key intermediate 9 displayed remarkable activity only against P. aeruginosa. Bioisosteric replacement of 4-one moiety with 4-thione resulted in compound 10 with a noticeable improvement in the antimicrobial profile. This might be attributed to the change in the electronic character as well as lipophilic properties of the molecule. Thus, a twofold increase in antimicrobial activity against S. aureus was observed (MIC =  $50 \mu g/mL$ ), whereas a remarkable increase in activity against E. coli and C. albicans was shown (MIC =  $12.5 \,\mu g/mL$  for both organisms). Substitution of the thione group with alkyl- or aralkylthio group resulted in three active compounds (11a-c). Among them, compound **11c** ( $R = CH_2CH_2-N(CH_2CH_3)_2$ ) showed the highest activity against *S. aureus* (MIC =  $12.5 \mu g/mL$ ), while compound **11b** ( $R = CH_2C_6H_5$ ) demonstrated the best activity against both P. aeruginosa and E. coli (MIC = 12.5  $\mu$ g/mL); whereas compound **11a** (R = CH<sub>3</sub>) exhibited substantial antifungal activity against C. albicans (MIC = 12.5  $\mu$ g/mL). Substitution of the methylthio group of **11a** with 4-chlorophenylamino 12b or benzylamino group 12c resulted in an altered spectrum of antimicrobial activity. Thus, compound 12b showed a twofold increase in activity against B. subtilis, while compound 12c displayed a twofold increase in activity against B. subtilis and P. aeruginosa. However, both compounds demonstrated a pronounced decrease in activity against E. coli and *C. albicans*. On the contrary, introduction of a phenyl or cyclohexyl amino group (12a, 12d) abolished the activ-



For **2**: **a**,  $X = OC_2H_5$ , **b**,  $X = NHC_6H_5$ , **c**,  $X = NHC_6H_4$ -Cl(4); for **4**: **a**,  $R = CH_3$ , **b**,  $R = C_6H_5$ ; for **5**: **a**,  $R^1 = H$ , **b**,  $R^1 = Cl$ ; for **6**: **a**,  $R^1 = H$ , **b**,  $R^1 = Cl$ 

**Reagents and conditions:** i) =  $H_2NSO_2C_6H_4NHNH_2 \times HCI / CH_3CO_2Na / EtOH, reflux; ii) = NH_2NH_2 \times H_2O / EtOH, reflux; iii) = RCOCI, pyridine, rt.; iv) = R'C_6H_4CHO / dioxane, reflux; v) = HSCH_2COOH / dioxane, reflux; vi) = CS_2 / KOH / EtOH, reflux; vii) = CH_3COCH_2COCH_3 / EtOH, reflux.$ 

Scheme 1. Synthesis of the intermediate and target compounds.

ity. The results also revealed that the hydrazine derivative **13** was found to possess considerable activity against *B. subtilis* and *P. aeruginosa*. Replacement of the hydrazino group with 5-amino-3-(4-chlorophenyl)pyrazolyl moiety **17b** or derivatization to 4-chlorophenylthiocarbamoylhydrazino **18b** led to an increase in both the antimicrobial potential and spectrum. Accordingly, compound **17b** displayed an eightfold increase in antimicrobial activity against *S. aureus* and *C. albicans* (MIC =  $12.5 \ \mu g/mL$ ), while compound **18b** demonstrated a twofold increase in activity against *P. aeruginosa* (MIC =  $50 \ \mu g/mL$ ) and an eightfold increase in activity against *C. albicans* (MIC =  $12.5 \ \mu g/mL$ ). Cyclization of compound **13** to the corresponding pyrazolotriazolopyrimidine **19** resulted in a dramatic increase in the antimicrobial activity against *S. aureus*, *B. subtilis*, and *P. aeruginosa* (MIC = 50,



For 11: a,  $R = CH_3$ , b,  $R = CH_2C_6H_5$ , c,  $R = CH_2CH_2N$ -(diethyl), d,  $R = CH_2CH_2$ -morpholino; for 12: a,  $R^{\dagger} = C_6H_5$ , b,  $R^{\dagger} = C_6H_4$ -Cl(4), c,  $R^{\dagger} = CH_2C_6H_5$ , d,  $R^{\dagger} = cyclohexyl$ .

Reagents and conditions: i) = HCONH<sub>2</sub>, reflux; ii) =  $P_2S_5$  / pyridine, reflux; iii) = RX / anhydrous K<sub>2</sub>CO<sub>3</sub> / DMF, rt.; iv) = R'NH<sub>2</sub>, heat at 160-170°C; v) = NH<sub>2</sub>NH<sub>2</sub> × H<sub>2</sub>O, EtOH, reflux.

Scheme 2. Synthesis of the intermediate and target compounds.

12.5, 12.5 μg/mL, respectively). Overall, compounds **2c**, **5b**, **10**, **11b**, **17b**, **18b**, and **19** was proven to be the most active antimicrobial compounds in this study.

## Conclusion

In conclusion, the results obtained from the preliminary *in-vitro* anticancer screening revealed that the pyrazolo[3,4-*d*]pyrimidine derivative **12a** was found to be the most active anticancer member in this study with a broad spectrum of activity against most of the tested subpanel tumor cell lines. According to the five-dose screen, compound **12a** showed particular effectiveness towards leukemia HL-60 (TB), K-562, non-small cell lung cancer NCI-H23, and colon cancer HT29, KM12 cell lines (GI<sub>50</sub> = 6.59, 4.44, 1.37, 3.33, and 9.63 µM, respectively). On the other hand, thirteen of the newly synthesized compounds displayed promising antibacterial activity against Gram-positive and Gram-negative bacteria with special high activity against P. aeruginosa in addition to a variable degree of antifungal activity against C. albicans. Potential antibacterial and antifungal activities were confined mainly to the pyrazolopyrimidine derivatives rather than the pyrazole ones. Compounds 2c, 5b, 10, 11b, 17b, 18b, and 19 were proven to be the most active antimicrobial members in this investigation with a broad spectrum of activity. Moreover, compounds 11b and 19 emerged with the highest potential against P. aeruginosa as they were four times superior to ampicillin. The best antifungal activity was demonstrated by compounds 2c, 5b, 10, 11a, 17b, and 18b which possessed half



For 16, 17 and 18: a, R = H, b, R = Cl

Reagents and conditions: i) CICOOEt, pyridine, reflux; ii) glacial CH<sub>3</sub>COOH, reflux; iii) R-C<sub>6</sub>H<sub>4</sub>CHO, dioxane, reflux; iv) R-C<sub>6</sub>H<sub>4</sub>COCH<sub>2</sub>CN, CH<sub>3</sub>COOH, EtOH, reflux; v) R-C<sub>6</sub>H<sub>4</sub>NCS, DMF, rt.; vi) CS<sub>2</sub>, pyridine, reflux or R-C<sub>6</sub>H<sub>4</sub>NCS, dioxane, reflux.

Scheme 3. Synthesis of the intermediate and target compounds.

the activity of clotrimazole. The results also pointed out that the 4-chloro derivatives **5b**, **12b**, **17b**, and **18b** elicited remarkable antimicrobial activity while their unsubstituted phenyl analogs were devoid of antimicrobial activity against all the tested organisms. Thus, it could be inferred that the presence of a chloro substituent imparts much towards the antibacterial power of this type of compounds. Finally, these types of compounds could be used as a fruitful template for further development of more potent and selective anticancer and / or antimicrobial agents.

For physicochemical and analytical data and IR and <sup>1</sup>H-NMR spectra of compounds **2–19** see Tables 6 and 7, respectively.

Compound	R, $\mathbb{R}^1$ or X	Yield (%)	M.p. (°C) (Crystallization solvent)*	Molecular Formula (Mol. weight) <sup>a)</sup>
2b	NHC <sub>6</sub> H <sub>5</sub>	62	315-316 (DMF / E)	C <sub>16</sub> H <sub>15</sub> N <sub>5</sub> O <sub>3</sub> S (357.39)
2c	$NH C_6H_4Cl(4)$	73	319–320 (DMF / E)	C <sub>16</sub> H <sub>14</sub> ClN <sub>5</sub> O <sub>3</sub> S (391.84)
3	-	82	296 – 297 (DMF / E)	C <sub>10</sub> H <sub>12</sub> N <sub>6</sub> O <sub>3</sub> S (296.31)
4a	$CH_3$	75	280-281 (DMF/E)	C <sub>12</sub> H <sub>14</sub> N <sub>6</sub> O <sub>4</sub> S (338.34)
4b	$C_6H_5$	65	277–278 (E)	$C_{17}H_{16}N_6O_4S$ (400.42)
5a	$C_6H_5$	78	300-301 (DMF/E)	$C_{17}H_{16}N_6O_3S(384.42)$
5b	$C_6H_4Cl(4)$	80	307-308 (DMF/E)	C <sub>17</sub> H <sub>15</sub> ClN <sub>6</sub> O <sub>3</sub> S (418.86)
6a	$C_6H_5$	50	257–258 (E)	$C_{19}H_{18}N_6O_4S_2$ (458.52)
6b	$C_6H_4Cl(4)$	51	273–274 (E)	C <sub>19</sub> H <sub>17</sub> ClN <sub>6</sub> O <sub>4</sub> S <sub>2</sub> (492.96)
7	-	84	268–270 (DMF/E)	$C_{11}H_{10}N_6O_3S_2$ (338.37)
8	-	86	244-245 (D)	C <sub>15</sub> H <sub>16</sub> N <sub>6</sub> O <sub>3</sub> S (360.39)
10	-	70	>320 (DMF / W)	$C_{11}H_9N_5O_2S_2$ (307.36)
11a	CH <sub>3</sub>	75	259–261 (D)	$C_{12}H_{11}N_5O_2S_2$ (321.38)
11b	$CH_2C_6H_5$	78	250-251 (D/W)	$C_{18}H_{15}N_5O_2S_2$ (397.48)
11c	$CH_2 CH_2 N(C_2 H_5)_2$	69	260-261 (D)	$C_{17}H_{22}N_6O_2S_2$ (406.53)
11d	CH <sub>2</sub> CH <sub>2</sub> -morpholino	73	236-237 (D)	$C_{17}H_{20}N_6O_3S_2$ (420.52)
12a	$C_6H_5$	65	>320 (DMF / E)	$C_{17}H_{14}N_6O_2S(366.41)$
12b	$C_6H_4Cl(4)$	67	>320 (DMF / E)	C <sub>17</sub> H <sub>13</sub> ClN <sub>6</sub> O <sub>2</sub> S (400.85)
12c	$CH_2C_6H_5$	74	250-251 (D)	$C_{18}H_{16}N_6O_2S(380.43)$
12d	cyclohexyl	62	263-264 (D)	C <sub>17</sub> H <sub>20</sub> N <sub>6</sub> O <sub>2</sub> S (372.45)
13	_	75	253-254 (DMF / W)	C <sub>11</sub> H <sub>11</sub> N <sub>7</sub> O <sub>2</sub> S (305.32)
14	-	68	>320 (DMF / E)	$C_{14}H_{15}N_7O_4S(377.38)$
15	-	74	>320 (DMF / W)	C <sub>13</sub> H <sub>11</sub> N <sub>7</sub> O <sub>2</sub> S (329.34)
16a	$C_6H_5$	72	>320 (DMF / E)	C <sub>18</sub> H <sub>15</sub> N <sub>7</sub> O <sub>2</sub> S (393.43)
16b	$C_6H_4Cl(4)$	75	>320 (DMF / E)	C <sub>18</sub> H <sub>14</sub> ClN <sub>7</sub> O <sub>2</sub> S (427.87)
17a	$C_6H_5$	63	306-307 (D)	C <sub>20</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> S (432.46)
17b	$C_6H_4Cl(4)$	65	>320 (D)	C <sub>20</sub> H <sub>15</sub> ClN <sub>8</sub> O <sub>2</sub> S (466.91)
18a	$C_6H_5$	80	>320 (DMF / E)	$C_{18}H_{16}N_8O_2S_2$ (440.51)
18b	$C_6H_4Cl(4)$	84	>320 (DMF / E)	C <sub>18</sub> H <sub>15</sub> ClN <sub>8</sub> O <sub>2</sub> S <sub>2</sub> (474.95)
19	_	73	>320 (DMF / W)	$C_{12}H_9N_7O_2S_2(347.38)$

Table 6. Physicochemical and analytical data for compounds 2-19.

\* Crystallization solvent (s): DMF (N,N-dimethylformamide), E: ethanol, W: water, D: dioxane.

 $^{\rm a)}$  The values found are within  $\pm$  0.4% of the calculated values.

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The authors have declared no conflict of interest.

## Experimental

#### Chemistry

Melting points were determined in open glass capillaries using Stuart Capillary melting point apparatus (Stuart Scientific Stone, Staffordshire, UK) and are uncorrected. Infrared (IR) spectra were recorded on Perkin-Elmer 1430 infrared spectrophotometer (Perkin Elmer, Beaconsfield, UK). <sup>1</sup>H-NMR spectra were scanned on Jeol-500 MHz spectrometer (Jeol, Tokyo, Japan) using tetramethylsilane (TMS) as internal standard and DMSO- $d_6$  as the solvent (chemical shifts are given in  $\delta$  ppm). Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Mass spectra were carried out using a Schimadzu GCMS-QP-1000EX mass spectrometer (Shimadzu, Tokyo, Japan) at 70 eV, Faculty of Science, Cairo University. Elemental analyses were performed at the microanalytical unit, Faculty of Science, Cairo University, and at the central lab, Faculty of Pharmacy, Alexandria University, Egypt and were found within ±0.4% of the theoretical values. Follow up of the reactions and checking the purity of the compounds was made by thin layer chromatography (TLC) on silica gel-precoated aluminium sheets (Type 60 GF<sub>254</sub>; Merck; Germany) and the spots were detected by exposure to UV lamp at  $\lambda_{254 \text{ nm}}$  for few seconds.

## 5-Amino-1-(4-sulfamoylphenyl)-N-(phenyl or 4chlorophenyl)-1H-pyrazole-4-carboxamides **2b**, **c**

A mixture of the selected *N*-substituted-2-cyano-3-ethoxyprop-2enamide (10 mmol), 4-hydrazinobenzenesulfonamide hydrochloride (2.24 g, 10 mmol) and anhydrous sodium acetate (0.82 g, 10 mmol) in ethanol (10 mL) was heated under reflux for 4 h, then allowed to attain room temperature. The separated solid product was filtered, washed with ethanol, dried, and crystallized. Physicochemical and analytical data are recorded in Tables 6 and 7.

## 4-[5-Amino-4-(hydrazinocarbonyl)-1H-pyrazol-1yl]benzenesulfonamide **3**

A suspension of 2a (6.2 g, 20 mmol) in hydrazine hydrate 80% (30 mL) was heated under reflux for 8 h. The reaction mixture

# Table 7. IR and <sup>1</sup>H-NMR spectra for compounds 2-19.

IR (KBr, cm <sup>-1</sup> )	<sup>1</sup> H-NMR (DMSO-d <sub>6</sub> )
<b>2b</b> , <b>c</b> : 3454 – 3421, 3370 – 3354, 3277 – 3273, 3180 – 3158 (NH <sub>2</sub> , NH), 1644 – 1626 (C = O), 1595 – 1592 (C = N), 1350 – 1329, 1164 – 1162 (SO <sub>2</sub> ).	<b>2c</b> : 6.72 (s, 2H, pyrazole NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.35, 7.71 (2d, J = 8.8 Hz, 4H, chlorophenyl-H), 7.48 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.77, 7.93 (2d, J = 8.8 Hz, 4H, sulfamoylphenyl C <sub>2.6</sub> -H & C <sub>3.5</sub> -H), 8.21 (s, 1H, pyrazole C <sub>3</sub> -H), 9.72 (s, 1H, NH, D <sub>2</sub> O exchangeable).
3: 3348, 3174 (NH <sub>2</sub> , NH), 1616 (C = O), 1597 (C = N), 1328, 1159 (SO <sub>2</sub> ).	<b>3</b> : 4.27 (s, 2H, hydrazine NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 6.51 (s, 2H, pyrazole NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.46 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.76 – 7.91 (2d, J = 8.03 Hz, 4H, benzenesulfonamide $C_{3,5}$ -H & $C_{2,6}$ -H), 7.92 (s, 1H, pyrazole $C_3$ -H), 9.18 (s, 1H, NH, D <sub>2</sub> O exchangeable).
<b>4a-c:</b> $3377 - 3375$ , $3339 - 3330$ , $3168 - 3164$ (NH <sub>2</sub> , NH), $1675 - 1672$ , $1649 - 1647$ (C = O), $1620 - 1619$ (C = N), $1320 - 1317$ , $1158 - 1157$ (SO <sub>2</sub> ).	<b>4b</b> : (s, 2H, pyrazole NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.47 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.48 – 7.58 (m, 3H, phenyl $C_{3,4,5}$ -H), 7.78 (d, J = 8.8 Hz, 2H, benzenesulfonamide $C_{3,5}$ -H), 7.89 (d, J = 7.65 Hz, phenyl $C_{2,6}$ -H), 7.93 (d, J = 8.8 Hz, 2H, benzenesulfonamide $C_{2,6}$ -H), 8.07 (s, 1H, pyrazole $C_3$ -H), 9.96, 10.33 (2s, 2H, 2NH, D <sub>2</sub> O exchangeable).
<b>5a, b</b> : 3424, 3292, 3216, 3190 (NH <sub>2</sub> , NH), 1643, 1614 (C = O), 1338, 1163 (SO <sub>2</sub> ).	<b>5b</b> : 6.74 (brs, 2H, NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.48 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.49, 7.69 (2d, J = 8.4 Hz, 4H, chlorophenyl-H), 7.78, 7.93 (2d, J = 8.8 Hz, 4H, benzenesulfona-mide $C_{3,5}$ -H & $C_{2,6}$ -H), 8.09 (s, 1H, pyrazole $C_3$ -H), 8.28 (s, 1H, N=CH), 11.38 (brs, 1H, NH, D <sub>2</sub> O exchangeable).
<b>6a, b</b> : 3445, 3341 (NH <sub>2</sub> , NH), 1685, 1653 (C = O), 1604 (C = N), 1339, 1165 (SO <sub>2</sub> ).	<b>6b</b> : 3.75, 3.92 (2d, J = 16.05 Hz, 2H, thiazolidine $C_5$ -H <sub>2</sub> ), 5.84 (s, 1H, thiazolidine $C_2$ -H), 6.62 (s, 2H, pyrazole NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.43 – 7.52 (m, 6H, chlorophenyl-H and SO <sub>2</sub> NH <sub>2</sub> ), 7.71, 7.90 (2d, J = 8.4 Hz, 4H, sulfamoylphenyl $C_{2,6}$ -H & $C_{3,5}$ -H), 7.84 (s, 1H, pyrazole $C_3$ -H), 10.15 (s, 1H, NH, D <sub>2</sub> O exchangeable).
<b>7</b> : 3397, 3318, 3291 (NH <sub>2</sub> ), 2731 (SH), 1630, 1594 (C = N), 1522, 1296, 1092, 946 (NCS), 1328, 1174 (SO <sub>2</sub> ).	<b>7</b> : 6.36 (s, 2H, pyrazole NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.49 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.76 (d, J = 8.8 Hz, 2H, benzenesulfonamide $C_{3,5}$ -H), 7.91 (s, 1H, pyrazole $C_3$ -H), 7.93 (d, J = 8.8 Hz, 2H, benzenesulfonamide $C_{2,6}$ -H), 14.59 (s, 1H, SH, D <sub>2</sub> O exchangeable).
<b>8</b> : 3441, 3345, 3187 (NH <sub>2</sub> ), 1656 (C = O), 1611 (C = N), 1337, 1164 (SO <sub>2</sub> ).	<b>8</b> : 2.20, 2.49 (2s, 6H, 2CH <sub>3</sub> ), 6.15 (s, 1H, pyrazole $C_4$ -H), 7.16 (s, 2H, pyrazole NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.50 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.76, 7.94 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$ -H & $C_{2,6}$ -H), 8.46 (s, 1H, pyrazole $C_3$ -H).
<b>10</b> : 3172, 3105 (NH <sub>2</sub> ), 2766 (SH), 1635 (C = N), 1539, 1301, 1093, 954 (NCS), 1337, 1154 (SO <sub>2</sub> ).	<b>10</b> : <b>7</b> .47 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> ), <b>7</b> .98, <b>8</b> .26 (2d, J = 8.4 Hz, 4H, benzenesulfonamide $C_{3,5}$ -H & $C_{2,6}$ -H), <b>8</b> .32 (s, 1H, pyrazolopyrimidine $C_{3}$ -H), <b>8</b> .48 (s, 1H, pyrazolopyrimidine $C_{6}$ -H), <b>13</b> .95 (s, 1H, SH, D <sub>2</sub> O exchangeable).
<b>11a-d</b> : 3341 – 3198 (NH <sub>2</sub> ), 1617 – 1593 (C = N), 1337 – 1332, 1155 – 1150 (SO <sub>2</sub> ), 1276 – 1272, 1095 – 1072 (C-S-C).	<b>11a</b> : 2.69 (s, 3H, CH <sub>3</sub> ), 7.47 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 8.0, 8.4 (2d, J = 8.4 Hz, 4H, benzenesulfonamide $C_{3,5}$ -H & $C_{2,6}$ -H), 8.64 (s, 1H, pyrazolopyrimidine $C_{3}$ -H), 8.88 (s, 1H, pyrazolopyrimidine $C_{6}$ -H). <b>11d</b> : 2.43 (t, J = 6.9 Hz, 2H, SCH <sub>2</sub> CH <sub>2</sub> N-), 2.65 (t, J = 6.9 Hz, 2H, SCH <sub>2</sub> CH <sub>2</sub> N), 3.52 (m, 8H, morpholine $C_{3,5}$ -H <sub>2</sub> & $C_{2,6}$ -H <sub>2</sub> ), 7.47 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 8.0, 8.4 (2d, J = 8.4 Hz, 4H, benzenesulfonamide $C_{3,5}$ -H & $C_{2,6}$ -H), 8.68 (s, 1H, pyrazolopyrimidine $C_{3}$ -H), 8.89 (s, 1H, pyrazolopyrimidine $C_{6}$ -H).
<b>12a-d</b> : 3380 – 3345, 3327 – 3293 (NH <sub>2</sub> ), 3198 – 3183 (NH), 1625 – 1615 (C = N), 1339, 1161 – 1155 (SO <sub>2</sub> ).	<b>12a</b> : 7.13 (t, J = 7.27 Hz, 1H, phenyl C <sub>4</sub> -H), 7.35 – 7.43 (m, 2H, phenyl C <sub>2.6</sub> -H), 7.45 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.82 (d, J = 7.27 Hz, 2H, phenyl C <sub>3.5</sub> -H), 7.99, 8.45 (2d, J = 8.77 Hz, 4H, benzenesulfonamide C <sub>3.5</sub> -H <sub>2</sub> & C <sub>2.6</sub> -H <sub>2</sub> ), 8.56 (s, 2H, pyrazolopyrimidine C <sub>3</sub> -H & pyrazolopyrimidine C <sub>6</sub> -H), 10.29 (s, 1H, NH, D <sub>2</sub> O exchangeable). <b>12c</b> : 4.76 (d, J = 6.1 Hz, 2H, CH <sub>2</sub> ), 7.21 – 7.36 (m, 5H, benzyl-H), 7.42 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.96, 8.43 (2d, J = 9.15 Hz, 4H, benzenesulfonamide C <sub>3.5</sub> -H & C <sub>2.6</sub> -H), 8.41 (s, 1H, pyrazolopyrimidine C <sub>3</sub> -H), 8.47 (s, 1H, pyrazolopyrimidine C <sub>6</sub> -H), 9.03 (t, J = 5.7 Hz, 1H, NH, D <sub>2</sub> O exchangeable).
<b>13</b> : 3313, 3207 (NH <sub>2</sub> , NH), 1584 (C = N), 1323, 1159 (SO <sub>2</sub> ).	<b>13</b> : 4.95 (s, 2H, NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.41 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.96 (d, J = 8.4 Hz, 2H, benzenesulfonamide $C_{3.5}$ -H), 8.22 (s, 1H, pyrazolopyrimidine $C_3$ -H), 8.45 (d, J = 8.4 Hz, 2H, benzenesulfonamide $C_{2.6}$ -H), 8.61 (s, 1H, pyrazolopyrimidine $C_6$ -H), 9.41 (s, 1H, NH, D <sub>2</sub> O exchangeable).
<b>14</b> : 3331, 3256, 3216 (NH <sub>2</sub> , NH), 1724 (C = O), 1590 (C = N), 1321, 1163 (SO <sub>2</sub> ), 1240, 1117, 1099 (C-O-C).	<b>14</b> : 1.21 (t, J = 6.9 Hz, 3H, CH <sub>3</sub> ), 4.1 (q, J = 6.9 ,Hz, 2H, CH <sub>2</sub> ), 7.45 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.98, 8.42 (2d, J = 8.4 Hz, 4H, sulfamoylphenyl C <sub>2.6</sub> -H & C <sub>3.5</sub> -H), 8.44 (s, 2H, pyrazolopyrimidine C <sub>3</sub> -H & pyrazolopyrimidine C <sub>6</sub> -H), 9.49, 10.04 (2s, 2H, 2NH, D <sub>2</sub> O exchangeable).

<b>Table 7</b> . Continue
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IR (KBr, cm <sup>-1</sup> )	<sup>1</sup> H-NMR (DMSO-d <sub>6</sub> )
<b>15</b> : 3288, 3186 (NH <sub>2</sub> ), 1648 (C = N), 1337, 1162 (SO <sub>2</sub> ).	<b>15</b> : 2.48 (s, 3H, CH <sub>3</sub> ), 7.51 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 8.02, 8.34 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3.5}$ -H & $C_{2.6}$ -H), 8.71 (s, 1H, pyrazolotriazolopyrimidine $C_{5}$ -H), 9.61 (s, 1H, pyrazolotriazolopyrimidine $C_{9}$ -H).
<b>16a, b</b> : $3361 - 3354$ , $3266 - 3211$ , $3202 - 3135$ (NH <sub>2</sub> , NH), $1612 - 1588$ (C = N), $1334 - 1321$ , $1160 - 1155$ (SO <sub>2</sub> ).	<b>16a</b> : 7.44 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.45 – 7.50 (m, 3H, phenyl $C_{3,4,5}$ -H), 7.79 (d, J = 6.9 Hz, 2H, phenyl $C_{2,6}$ -H), 7.99 (d, J = 9.15 Hz, 2H, benzenesulfonamide $C_{3,5}$ -H), 8.27 (s, 1H, N=CH), 8.46 (d, J = 9.15 Hz, 2H, benzenesulfonamide $C_{2,6}$ -H), 8.50 (s, 1H, pyrazolopyrimidine $C_3$ -H), 8.65 (s, 1H, pyrazolopyrimidine $C_6$ -H), 12.30 (s, 1H, NH, D <sub>2</sub> O exchangeable).
<b>17a</b> , <b>b</b> : $3443 - 3424$ , $3372 - 3341$ , $3216 - 3210$ , $3190 - 3175$ (NH <sub>2</sub> , NH), $1643 - 1639$ , $1614 - 1595$ (C = N), $1338 - 1337$ , $1163 - 1161$ (SO <sub>2</sub> ).	<b>17b</b> : 5.94 (s, 1H, pyrazole $C_{4}$ -H), 7.44 (s, 2H, NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.47 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.48, 7.98 (2d, J = 7.25 Hz, 4H, chlorophenyl-H), 8.03, 8.44 (2d, J = 8.8 Hz, 4H, benzenesulfonamide $C_{3,5}$ -H & $C_{2,6}$ -H), 8.89 (s, 1H, pyrazolopyrimidine $C_{3}$ -H), 9.0 (s, 1H, pyrazolopyrimidine $C_{6}$ -H)
$\begin{array}{l} \textbf{18a, b: } 3318 - 3312, 3242 - 3229, 3140 - 3145 \\ (NH_2, NH), 1592 - 1591 (C = N), 1329 - 1321, \\ 1157 - 1151 (SO_2), 1536 - 1516, 1274 - 1273, \\ 1094 - 1093, 969 - 944 (NCS). \end{array}$	<b>18a:</b> 7.08 – 7.38 (m, 5H, phenyl-H), 7.44 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 7.99 (d, J = 8.8 Hz, 2H, benzenesulfonamide $C_{3,5}$ -H), 8.35 (s, 1H, pyrazolopyrimidine $C_3$ -H), 8.45 (d, J = 8.8 Hz, 2H, benzenesulfonamide $C_{2,6}$ -H), 8.55 (s, 1H, pyrazolopyrimidine $C_6$ -H), 10.19, 10.35, 10.52 (3s, 3H, 3NH, D <sub>2</sub> O exchangeable).
<b>19</b> : 3366, 3267 (NH <sub>2</sub> ), 2792 (SH), 1593 (C = N), 1520, 1293, 1098, 965 (NCS), 1335, 1157 (SO <sub>2</sub> ).	7.51 (s, 2H, SO <sub>2</sub> NH <sub>2</sub> , D <sub>2</sub> O exchangeable), 8.02, 8.24 (2d, J = 8.4 Hz, 4H, benzenesulfona- mide C <sub>3.5</sub> -H & C <sub>2.6</sub> -H), 8.66 (s, 1H, pyrazolotriazolopyrimidine C <sub>5</sub> -H), 9.03 (s, 1H, pyrazo- lotriazolopyrimidine C <sub>5</sub> -H), 14.64 (brs, 1H, SH, D <sub>2</sub> O exchangeable).

was left overnight and the separated solid was filtered, washed with ethanol, dried and recrystallized. Physicochemical and analytical data are recorded in Tables 6 and 7.

#### 4-{[4-(2-Acetyl or benzoylhydrazinocarbonyl)]-5-Amino-1H-pyrazol-1-yl}benzensulfonamides **4a**, **b**

A mixture of **3** (1 g, 3.38 mmol) and acetyl chloride or benzoyl chloride (3.38 mmol) in dry pyridine (5 mL) was stirred at room temperature for 6 h. The reaction mixture was poured onto crushed ice and the separated solid was filtered off, washed with water, dried, and crystallized. Physicochemical and analytical data are recorded in Tables 6 and 7.

## 5-Amino-N-[4-oxo-2-(phenyl or 4-chlorophenyl)-3thiazolidin-3-yl]-1-(4-sulfamoylphenyl)-1H-pyrazole-4carboxamides **6a**, **b**

A mixture of **5a**, **b** (1 mmol) and thioglycolic acid (2.65 g, 2 mL, 29 mmol) in dry dioxane (30 mL) was heated under reflux for 18 h. The solvent was evaporated under reduced pressure and then neutralized with 10% sodium carbonate solution. The solid was filtered off, washed with water, dried, and crystallized. Physicochemical and analytical data are recorded in Tables 6 and 7.

#### 4-{5-Amino-4-[(3,5-dimethyl-1H-pyrazol-1-yl)carbonyl]-1H-pyrazol-1-yl}benzenesulfonamide **8**

A mixture of equimolar amounts of **3** (0.6 g, 2 mmol) and acetyl acetone (0.2 g, 2 mmol) in absolute ethanol (15 mL) was refluxed for 8 h, then allowed to attain room temperature. The separated crystalline product was filtered, dried, and recrystallized. Physicochemical and analytical data are recorded in Tables 6 and 7.

#### 4-(4-Substituted amino-1H-pyrazolo[3,4-d]pyrimidin-1vl)benzenesulfonamides **12a-d**

A mixture of **11a** (1 g, 3 mmol) and the selected amine (9 mmol) was heated in an oil bath at  $160-170^{\circ}$ C for 6 h. The reaction mixture was triturated with dilute hydrochloric acid, filtered, washed with water, dried, and crystallized. Physicochemical and analytical data are recorded in Tables 6 and 7.

## 4-(4-Hydrazino-1H-pyrazolo[3,4-d]pyrimidin-1yl)benzenesulfonamide **13**

To a suspension of **11a** (10 g, 30 mmol) in ethanol (50 mL), hydrazine hydrate (12 g, 11.6 mL, 240 mmol) was added and the reaction mixture was heated under reflux for 6 h. It was then left to cool and the obtained product was filtered, washed with ethanol, dried, and crystallized. Physicochemical and analytical data are recorded in Tables 6 and 7.

# *Ethyl 2-[1-(4-sulfamoylphenyl)-1H-pyrazolo[3,4d]pyrimidin-4-yl]hydrazinecarboxylate* **14**

To a suspension of the hydrazine **13** (1 g, 3.3 mmol) in dry pyridine (5 mL), ethyl chloroformate (0.43 g, 0.38 mL, 4 mmol) was added dropwise while stirring in an ice bath. The reaction mixture was left stirred at room temperature for 2 h, and then heated under reflux for further 2 h. It was then allowed to cool, poured onto crushed ice and the obtained product was filtered, washed with water, dried, and crystallized. Physicochemical and analytical data are recorded in Tables 6 and 7.

## 4-(3-Methyl-7H-pyrazolo[4,3-e][1,2,4]triazolo[4,3c]pyrimidin-7-yl)benzenesulfonamide **15**

A suspension of **13** (1 g, 3.3 mmol) in glacial acetic acid (8 mL) was heated under reflux for 6 h then left to cool to room temper-

ature. The separated product was filtered, washed with ethanol, dried, and crystallized. Physicochemical and analytical data are recorded in Tables 6 and 7.

#### 4-[4-(5-Amino-3-aryl-1H-pyrazol-1-yl)-1H-pyrazolo[3,4d]pyrimidin-1-yl]benzenesulfonamides **17a**, **b**

To a suspension of **13** (1 g, 3.3 mmol) in ethanol / acetic acid mixture (4:1) (20 mL), the appropriate phenacyl cyanide (3.3 mmol) was added. The reaction mixture was heated under reflux for 6 h during which a crystalline precipitate separated out. The reaction mixture was cooled, filtered, washed with ethanol, and crystallized. Physicochemical and analytical data are recorded in Tables 6 and 7.

## 4-[4-(N-Substituted thiocarbamoylhydrazino)-1Hpyrazolo[3,4-d]pyrimidin-1-yl]benzenesulfonamides **18a. b**

To a solution of the hydrazine **13** (1 g, 3.3 mmol) in dry DMF (5 mL), the appropriate arylisothiocyanate (3.3 mmol) was added and the mixture was stirred at room temperature for 6 h. The reaction mixture was poured onto crushed ice and the separated solid product was filtered, washed with water, dried, and crystallized. Physicochemical and analytical data are recorded in Tables 6 and 7.

## 4-(3-Sulfanyl-7H-pyrazolo[4,3-e][1,2,4]triazolo[4,3c]pyrimidin-7-yl)benzenesulfonamide **19**

To a solution of **13** (0.6 g, 2 mmol) in dry pyridine (5 mL), carbon disulfide (3.8 g, 3 mL, 50 mmol) was added and the mixture was heated under reflux for 8 h. The reaction mixture was poured onto crushed ice and the separated solid product was filtered, washed with water, dried, and crystallized. Physicochemical and analytical data are recorded in Tables 6 and 7.

#### Biology

#### Anticancer screening

Fourteen of the prepared compounds were selected by the National Cancer Institute (NCI) and tested for their *in-vitro* anticancer activity against 60 human tumor cell lines, derived from nine clinically isolated types of cancer (leukemia, lung, brain, melanoma, colon, ovarian, renal, breast, and prostate). These cell lines were incubated with one concentration (10  $\mu$ M) for each tested compound. Only compounds which satisfy pre-determined threshold-inhibition criteria were tested at five tenfold dilutions (0.01 to 100  $\mu$ M). A 48 h continuous drug-exposure protocol was used, and a sulforhodamine B (SRB) protein assay was employed to estimate the cell viability or growth [40, 41]. The results are presented in Tables 1 and 2.

#### Antimicrobial screening

#### Inhibition-zone measurements

All the synthesized compounds were evaluated by the agar cup diffusion technique [43] using a 1 mg/mL solution in DMSO. The test organisms were *Staphylococcus aureus* (DSM 1104) and *Bacillus subtilis* (ATCC 6633) as Gram-positive bacteria; *Escherichia coli* (ATCC 11775) and *Pseudomonas aeruginosa* (ATCC 10145) as Gramnegative bacteria. *Candida albicans* (DSM 70014) was also used as a representative for fungi. Each 100 mL of sterile molten agar (at 45°C) received 1 mL of 6 h-broth culture and then the seeded

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agar was poured into sterile Petri dishes. Cups (8 mm in diameter) were cut in the agar. Each cup received 0.1 mL of the 1 mg/ mL solution of the test compounds. The plates were then incubated at  $37^{\circ}$ C for 24 h or, in case of *C. albicans*, for 48 h. A control using DMSO without the test compound was included for each organism. Ampicillin was used as standard antibacterial, while clotrimazole was used as antifungal reference. The resulting inhibition zones are recorded (Table 4).

#### Minimal inhibitory concentration (MIC) measurement

The minimal inhibitory concentrations (MIC) of the most active compounds were measured using the twofold serial broth dilution method [44]. The test organisms were grown in their suitable broth: 24 h for bacteria and 48 h for fungi at  $37^{\circ}$ C. Twofold serial dilutions of solutions of the test compounds were prepared using 200, 100, 50, 25, and 12.5 µg/mL. The tubes were then inoculated with the test organisms; each 5 mL received 0.1 mL of the above inoculum and were incubated at  $37^{\circ}$ C for 48 h. Then, the tubes were observed for the presence or absence of microbial growth. The MIC values of the prepared compounds are listed in Table 5.

#### Minimal bactericidal concentration (MBC) measurement

MIC tests were always extended to measure the MBC as follows: A loop-full from the tube not showing visible growth (MIC) was spread over a quarter of Müller–Hinton agar plate. After 18 h of incubation, the plates were examined for growth. Again, the tube containing the lowest concentration of the test compound that failed to yield growth on subculture plates was judged to contain the MBC of that compound for the respective test organism (Table 6).

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