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Synthesis and initial biological evaluation of substituted 1-phenylamino-2-thio-4,5-dimethyl-1*H*-imidazole derivatives

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ABSTRACT

In this work, some new 2-[(4,5-dimethyl-1-(arylamino)-1*H*-imidazol-2-yl)thio]-1-(aryl)ethanone derivatives were synthesized and investigated for their antibacterial, antifungal and anticancer activities. Toxicity of the most effective compounds was established by performing Brine-Shrimp lethality assay. Antifungal activity of the compounds was found to be higher than antibacterial and anticancer activities of the compounds.

In general, imidazoles and azoles are important family of heterocyclic compounds with a broad interest due to their bioactive properties. Various imidazole derivatives have been reported with a broad range of bioactivities, such as antifungal, antiprotozoal, antiinflammatory, antiallergic, antihistaminic, antiulcer, antihelmintic, analgesic, antihypertensive, antineoplastic activity and neuroleptic antipsychotic and thromboxane synthetase inhibitory activity.[1–4] It is well-known that the azole scaffold is a major chemical group with antifungal activity acting as a pharmacophoric group. Imidazole derivatives (clotrimazole, econazole, miconazole, misonidazole, metranidazole, ketoconazole, itraconazole, fluconazole, fenticonazole) control fungal infections by blocking ergosterol biosynthesis which is an essential component of fungal cell wall, causing its depletion and accumulation of lanosterol and some other 14-methylsterols.[5–8] Imidazole ring is also present in some of the clinically used drug structures (asetomidate, cimetidine, omeprazole, lansoprazole, azomycine, flumazenil, thyroliberin, methimazole, pilocarpine and etomidate) acting as a pharmacophoric group or a substituent (space).[9,10] Although there is a lot of imidazole including drugs, the activity is not always directly related with the imidazole structure.

Imidazoles form the main structure of some well-known biomolecules of human organisms, such as the amino acid histidine, vit B_{12}, histamine and biotin.[11] Ribotide 4(5)-aminoimidazol-5(4)-carboxamide is an another imidazole containing biomolecule which is a key compound in the biosynthesis of natural purine component of RNA and DNA. Imidazole moiety containing compounds are also known as antimetabolite drugs.[12,13] Imidazole-containing anticancer drugs currently undergoing clinical studies are known to act by the same mechanism as mercaptopurine;[14] imidazolfurin which is evaluated for its ability to inhibit the growth of human myelogenous leukemia K562 cells;[15] thioguanine which is an antimetabolite classically used as an alternative drug in children with acute lymphoblastic leukaemia;[16] cytarabine a nucleoside antimetabolite frequently used in the treatment of hematological malignancies;[17] pentostatin, a purine nucleoside analog, which is highly efficacious in the treatment of many indolent lymphoproliferative disorders, including hairy-cell leukemia, chronic lymphocytic leukemia, and low-grade non-Hodgkin's lymphomas.[18]

Motivated by the above data and as an extension of our previous works,[19,20] we attempted to prepare a new series of compounds containing imidazole structure as the pharmacophoric group.

Forty new imidazole compounds (**1**–**40**) were synthesized by two steps. The synthesis pathway of the compounds is shown in Figure 1.[21] and all the synthesized compounds are listed in Table 1. The structures of the synthesized compounds were confirmed by means of ^{1}H and ^{13}C NMR spectra, while the molecular weights of the compounds were confirmed by molecular ions detected by

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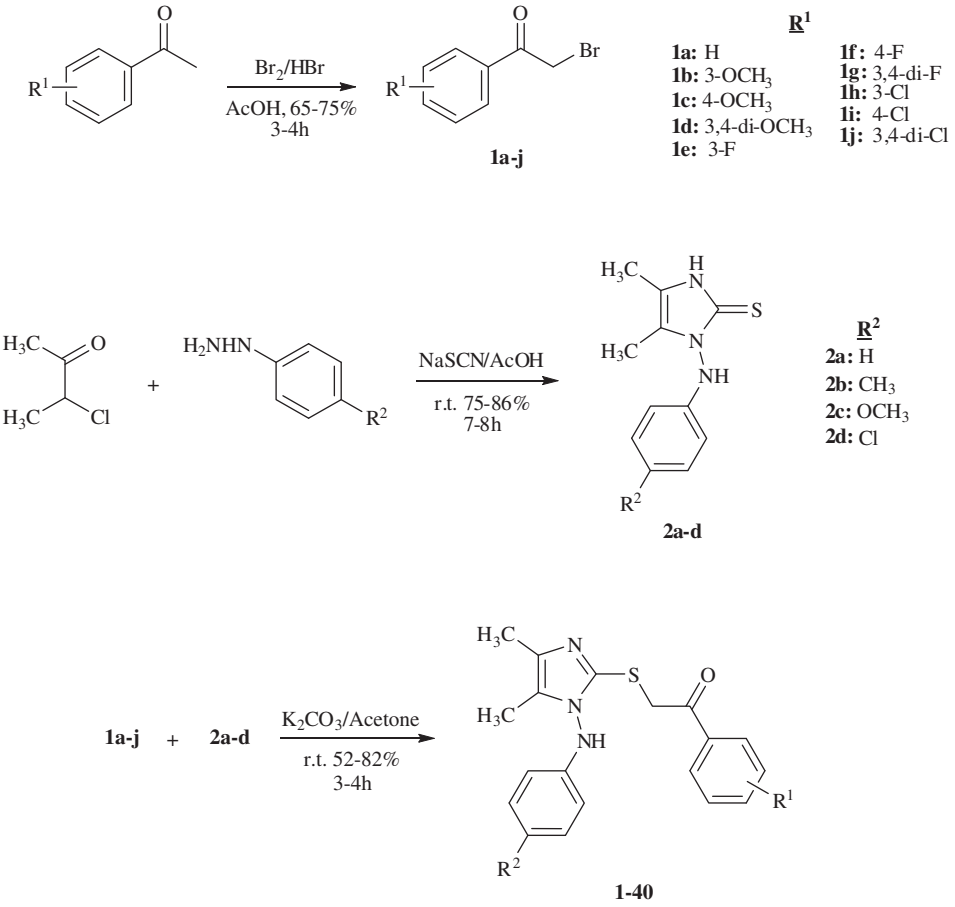

Figure 1. The synthetic protocol of the compounds, reagents and conditions (**1**–**40**).

FAB. Additionally, elemental analyses of all compounds gave satisfactory results as cited in the Supplementary data.

The synthesized new imidazole compounds were screened for in vitro antimicrobial activity against four species of Gram-positive bacteria, *Enterecoccus faecalis* (ATCC 29212), *E. faecalis* (ATCC 51299), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19115), and four Gram-negative bacteria, *Escherichia coli* (ATCC 25922), *E. coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 13883) and three candida species *Candida albicans* (ATCC 90028), *C. glabrata* (ATCC 90030), *C. krusei* (ATCC 6258). MIC is defined as the lowest concentration of the compounds that completely inhibited microbial growth after 24 h incubation at 35 °C. MIC values of the synthesized compounds were given along with the reference drugs chloramphenicol and ketoconazole.

As shown in Table 2, the tested imidazole derivatives exhibited varying degrees of inhibitory effects on the growth of selected microbial strains. In general, most of the compounds were more active against Gram-positive bacteria than Gram-negative bacteria. The Gram-positive bacterium *L. monocytogenes* (ATCC 19115) was found to be the most susceptible strain. Against *L. monocytogenes* (ATCC 19115), the compounds **7**, **13**, **14**, and **37** were equipotent to chloramphenicol (MIC = 50 μg/mL). Compounds **37** and **40** (MIC = 50 μg/mL) were found to be four times less active than chloramphenicol, while compounds **7** and **13** (MIC = 25 μg/mL) showed half activity to chloramphenicol against *E. faecalis* (ATCC 29212). Compounds **17**, **21** and **22** were most active against *E. faecalis* (ATCC 51255) with MIC values 200, 100, and 200 μg/mL, respectively, whereas the MIC value for chloramphenicol was 100 μg/mL. Compound **7** had the highest activity against *S. aureus* (ATCC 25923) (MIC = 100 μg/mL). The MIC values observed for Gram-negative bacteria were in a similar range, with compound **13** being half as potent as the standard against *E. coli* (ATCC 25922). Compounds **4**, **11**, **12**, **14** and **40** showed some activity against *E. coli* (ATCC 35218), similar to compounds **1** and **3** when tested against *P. aeruginosa*. Another Gram-negative bacterium, *K. pneumonia* (ATCC 13883) was not inhibited by any of the compounds reported here. Also, compounds **16** and **36** lacked antimicrobial activity against all the tested strains.

The antifungal activity of the compounds was studied against three Candida species, the most sensitive *Candida* was established as *C. krusei* (ATCC 6258) and compounds **13**, **14** and **37** were found to be the most active antifungal agent. Against *C. albicans* (ATCC 90028) compounds **13** (MIC = 25 μg/mL), **14** (MIC = 100 μg/mL) and **37** (MIC = 50 μg/mL) were determined as the most active compounds. These three compounds showed antifungal activity similar to ketoconazole with MIC value of 100 μg/mL against *C. glabrata* (ATCC 90030). Compounds **4** and **14** (MIC = 50 μg/mL) displayed same activity of ketoconazole; meanwhile compound **13** (MIC = 25 μg/mL) showed activity two times better than ketoconazole.

The imidazole compounds described herein belong to four structurally related families of derivatives that differ in the substitution pattern on the phenylamino moiety (R^2 = H, CH_3, OCH_3, and Cl). Within each of these four series, various substitution patterns on the acetophenone phenyl ring (R^1 = H, 3-OMe, 4-OMe etc.,) were introduced. A comparison of the properties induced by substitution on the aminophenyl moiety revealed that antimicrobial activities increased for the methyl- and the chloro- substitution, irrespective of electron donating/withdrawing properties of these groups. This

Table 1
List of the synthesized compounds (**1**–**40**) and reaction conditions

Compound	R^1	R^2	Time (h)	yield (%)
1	H	H	3–4	82
2	3-OCH_3	H	3–4	69
3	4-OCH_3	H	3–4	73
4	3,4-Di-OCH_3	H	3–4	77
5	3-F	H	3–4	62
6	4-F	H	3–4	71
7	3,4-Di-F	H	3–4	72
8	3-Cl	H	3–4	75
9	4-Cl	H	3–4	68
10	3,4-Di-Cl	H	3–4	77
11	H	CH_3	3–4	73
12	3-OCH_3	CH_3	3–4	75
13	4-OCH_3	CH_3	3–4	78
14	3,4-Di-OCH_3	CH_3	3–4	80
15	3-F	CH_3	3–4	52
16	4-F	CH_3	3–4	67
17	3,4-Di-F	CH_3	3–4	68
18	3-Cl	CH_3	3–4	71
19	4-Cl	CH_3	3–4	64
20	3,4-Di-Cl	CH_3	3–4	60
21	H	OCH_3	3–4	62
22	3-OCH_3	OCH_3	3–4	65
23	4-OCH_3	OCH_3	3–4	70
24	3,4-Di-OCH_3	OCH_3	3–4	75
25	3-F	OCH_3	3–4	74
26	4-F	OCH_3	3–4	72
27	3,4-Di-F	OCH_3	3–4	69
28	3-Cl	OCH_3	3–4	64
29	4-Cl	OCH_3	3–4	77
30	3,4-Di-Cl	OCH_3	3–4	78
31	H	Cl	3–4	77
32	3-OCH_3	Cl	3–4	79
33	4-OCH_3	Cl	3-4	67
34	3,4-Di-OCH_3	Cl	3-4	71
35	3-F	Cl	3–4	70
36	4-F	Cl	3–4	68
37	3,4-Di-F	Cl	3–4	66
38	3-Cl	Cl	3–4	78
39	4-Cl	Cl	3–4	69
40	3,4-Di-Cl	Cl	3–4	73

was manifested especially in the activities of the methyl derivatives ($R^2 = CH_3$) against the *Candida* species. Thus, compounds **7**, **13**, **14**, **33**, **34**, and **37** were the most active. In addition, we observed that 4-methoxy, 3,4- dimethoxy, and 3,4-difluoro substitution (R^1 = 4-OMe; 3,4-di-OMe; 3,4-di-F) all had a beneficial effect on the observed antimicrobial activities. Interestingly, the 4-fluoro substituted compounds **6**, **16**, **26** and **36** (R^1 = 4-F) were not active, while the 3,4-difluoro analogues (R^1 = 3,4-di-F) were active. A complex interplay of electronic and other effects may be responsible for these observed differences.

Cytotoxicity in brine shrimp lethality test was studied in order to reveal new antimicrobial and anticancer compounds. This assay is regarded as a useful method for preliminary evaluation of toxicity which is also used for antitumor, pesticidal and antitrypanosomal activity evaluation.[22] Toxicity test was analysed with the LC50 computer program (Trimmed Spearman–Karber Method, Version 1.5) so as to calculate LC50 values and 95% confidence intervals.[23] LC50 values of the compounds **7**, **13** and **37** (465.05, 152.74 and 359.21 μg/mL) were found between 100 μg/mL and 500 μg/mL and categorized as having moderate cytotoxicity.[24] LC50 values greater than 1000 μg/ml were considered to be non toxic. Accordingly, compounds **1** and **14** were appreciated as non-toxic. Toxicity test results were presented in Table 3.

Compound **1** (NSC 772200), **3** (NSC 772201) and **9** (NSC 722202) were selected by NCI for 60 human tumor cell lines' anticancer screening test at single dose assay. Results were given as percentage growth of tumor cells which were treated with selected three

Table 2
Antimicrobial activity of the compounds **1**–**40** (μg/mL)

Comp	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
1	800	800	200	800	800	800	800	800	400	800	400
2	400	800	400	400	800	800	800	800	400	400	400
3	400	800	200	800	400	800	800	800	400	800	800
4	800	200	400	400	400	800	200	400	200	800	50
5	800	400	400	400	400	400	400	400	400	400	200
6	800	400	400	400	400	400	800	400	400	400	200
7	400	400	400	400	25	400	100	50	400	400	200
8	400	400	400	800	400	400	400	400	800	400	200
9	800	400	400	800	200	400	800	400	200	400	200
10	800	800	400	800	200	800	800	400	400	200	200
11	800	200	800	400	400	800	800	800	800	400	400
12	800	200	800	800	800	400	800	400	400	400	200
13	100	400	400	400	25	400	400	50	25	100	25
14	200	200	400	400	100	400	400	50	100	100	50
15	800	800	400	400	200	400	800	400	800	800	400
16	1600	1600	1600	1600	1600	1600	1600	1600	1600	1600	1600
17	800	800	800	800	400	200	800	800	200	400	100
18	800	800	800	800	800	800	800	800	800	800	800
19	400	800	800	800	400	400	800	800	400	400	200
20	400	800	800	800	400	400	800	800	800	800	800
21	800	800	800	800	800	100	800	800	800	800	800
22	800	800	800	800	800	200	800	800	800	800	800
23	800	800	800	800	800	800	800	800	400	800	400
24	800	800	800	800	800	1600	800	800	200	400	200
25	800	800	400	800	400	800	400	400	400	400	400
26	800	800	800	800	1600	800	800	400	400	400	400
27	800	800	800	800	1600	800	800	800	400	800	400
28	800	800	800	800	800	800	800	800	800	800	400
29	400	800	800	400	400	800	400	400	400	400	400
30	400	800	400	800	400	800	800	800	800	800	800
31	800	800	400	800	200	800	800	400	800	800	800
32	800	800	800	400	200	400	800	1600	800	800	200
33	800	800	800	400	200	400	400	1600	800	800	200
34	400	800	400	800	100	800	800	100	400	400	400
35	400	800	400	800	100	800	800	200	400	400	400
36	1600	1600	1600	800	1600	800	800	3200	800	1600	800
37	400	800	400	400	50	400	400	50	50	100	100
38	800	800	400	800	400	800	800	800	800	400	800
39	800	800	400	800	800	800	800	800	800	800	400
40	800	200	400	800	50	800	800	200	800	400	400
Ref. 1	50	25	50	50	12.5	100	50	50	—	—	—
Ref. 2	—	—	—	—	—	—	—	—	100	100	50

Ref. 1: Chloramphenicol, Ref. 2: Ketoconazole.
A: *E. coli* (ATCC 25922), **B**: *E. coli* (ATCC 35218), **C**: *P. aeruginosa* (ATCC 27853), **D**: *K. pneumonia* (ATCC 13883), **E**: *E. faecalis* (ATCC 29212), **F**: *E. faecalis* (ATCC 51299), **G**: *S. aureus* (ATCC 25923), **H**: *L. monocytogenes* (ATCC 19115), **I**: *C. albicans* (ATCC 90028), **J**: *C. glabrata* (ATCC 90030),**K**: *C. krusei* (ATCC 6258).

compounds (Table 4). The expected potential anticancer activity could not be observed from the compounds. Among three tested compounds compound **3** was assigned as the most active one and among the sixty tumor cell line UO-31 which is derived from renal cancer was found to be the most sensitive cell line against three tested compounds (compounds **1**, **3** and **9**) with the growth percentages 68.60%, 69.91% and 66.15%, respectively.

In summary, the objective of the present study was the synthesis of 2-[(4,5-dimethyl-1-(substituted phenylamino)-1*H*-imidazol-2-yl)thio]-1-(substituted phenyl)ethanone derivatives (**1**–**40**) and evaluation of their antibacterial, antifungal and anticancer activities. Compounds **13**, **14** and **37** displayed significant antifungal activity warranting further attention. Antibacterial activity of the compounds was higher against Gram-positive bacteria than Gram-negative bacteria and a Gram-positive bacterium *L. monocytogenes* (ATCC 19115) was found to be the most susceptible bacterium. According to toxicity test results, our compounds were found to be non-toxic or have little toxicity in the brine shrimp test. From the comparison of cytotoxicity and antimicrobial activity test results, it might be claimed that

Table 3
Brine-shrimp toxicity results of the compounds **1**, **7**, **13**, **14** and **37**

Concentration (μg/mL)	**1**	**7**	Mortality[a] **13**	**14**	**37**
1600	3	9	10	6	10
800	3	7	10	3	10
400	2	5	10	2	5
200	1	3	7	0	2
100	0	2	3	0	0
50	0	2	1	0	0
Control	1	1	1	1	1
LC50	>1600	465.05	152.74	1307.13	359.21
95% CI	—	290.42–741.48	115.96–201.20	769.03–2221.75	272.95–472.73

[a] Ten organisms (*Artemia salina*) tested for each concentration. The results are presented as LC50 values (μg/mL) and 95% confidence intervals (CI).

Table 4
60 Human tumor cell lines' anticancer screening data at single dose assay as percent cell growth promotion of selected compounds

Cell lines	Compounds		
	1	**3**	**9**
L			
CCRF-CEM	90.27	89.68	81.64
HL-60(TB)	91.00	87.13	92.02
K-562	89.68	87.79	88.10
MOLT-4	100.2	89.96	87.31
RPMI-8226	102.2	94.68	92.33
SR	82.35	83.40	86.04
NSCLC			
A549/ATCC	99.16	97.35	86.67
HOP-62	97.72	100.7	101.2
HOP-92	91.79	82.74	76.51
NCI-H226	99.07	92.08	95.15
NCI-H23	93.43	88.38	89.25
NCI-H322M	90.31	82.37	88.63
NCI-H460	104.4	96.54	98.85
NCI-H522	81.35	77.18	79.92
CC			
COLO 205	110.9	100.5	96.15
HCC-2998	110.5	93.49	106.9
HCT-116	86.93	96.71	90.70
HCT-15	99.30	88.63	92.65
HT29	97.65	94.25	99.26
KM12	104.6	97.97	105.0
SW-620	109.1	105.7	108.9
CNSC			
SF-268	101.9	104.5	103.9
SF-295	86.98	90.39	89.88
SF-539	104.4	100.8	103.9
SNB-19	98.31	96.63	89.00
SNB-75	108.2	93.05	94.11
U251	97.26	93.77	90.68
M			
LOX IMVI	102.1	104.8	101.4
MALME-3M	103.5	101.4	97.11
M14	101.1	91.19	107.4
MDA-MB-435	97.36	95.25	99.36
SK-MEL-2	97.16	94.21	91.02
SK-MEL-28	110.7	113.1	108.7
SK-MEL-5	99.80	100.7	96.98
UACC-257	97.59	99.75	97.88
UACC-62	102.6	90.38	90.32
OC			
IGROV1	98.59	103.3	102.1
OVCAR-3	109.4	101.3	108.6
OVCAR-4	105.2	92.83	93.89
OVCAR-5	109.7	99.29	103.9
OVCAR-8	99.29	100.7	96.67
NCI/ADR-RES	96.87	83.61	93.39
SK-OV-3	97.79	93.26	102.4
RC			
786-0	106.4	90.03	97.67
A498	91.12	90.87	82.14
ACHN	106.6	99.05	102.7
CAKI-1	88.11	81.41	78.89
RXF 393	103.1	85.48	96.88
SN12C	99.90	98.88	93.18
TK-10	127.6	109.3	117.2
UO-31	68.60	69.91	66.15
PC			
PC-3	102.0	89.35	91.86
DU-145	109.3	111.6	115.8
BC			
MCF7	96.90	105.7	88.08
MDA-MB-231/ATCC	98.84	89.05	95.48
HS 578T	108.0	107.2	96.61
BT-549	88.93	84.66	104.7
T-47D	85.54	71.62	79.51
Mean	98.94	94.06	95.05
MCF7	96.90	105.7	88.08
Delta	30.34	24.15	28.90
Range	58.95	43.14	51.07

compounds **13**, **14** and **37** have antimicrobial activity because of their selective antimicrobial effects not because of their general toxicities; as it should be antimicrobial agents. Findings from the activity and toxicity studies have encouraged the achievement of the synthesis of new similar compounds (more selective, more active and non-toxic derivatives) in ongoing studies. Additionally, it will be interesting to study the mechanism of the action for further investigations.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.10.024.

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