SYNTHESIS AND ANTICANCER EVALUATION OF NEW BENZENESULFONAMIDE DERIVATIVES

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A highly efficient protocol was developed for the synthesis of 3-(indoline-1-carbonyl)-N-(substituted)benzenesulfonamide compounds with excellent yields. The in vitro anticancer activity of the new 3-(indoline-1-carbonyl)-N-(substituted)benzenesulfonamide derivatives against A549 (lung cancer cell), HeLa (cervical), MCF-7 (breast cancer cell) and Du-145 (prostate cancer cell) cell lines were studied. Most of the tested compounds showed anticancer activity (IC_{50} values ranged between 1.98 and 9.12 µM against different cell lines).

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INTRODUCTION

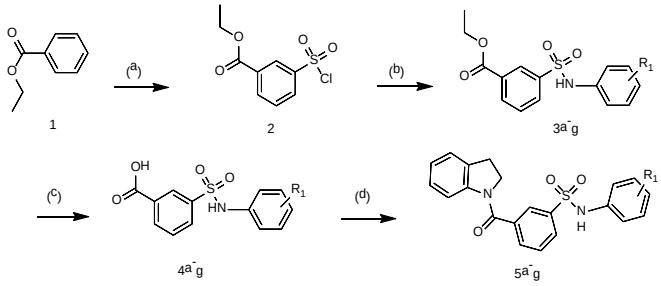
Antibiotic-resistant bacteria are rapidly emerging worldwide.[1] The indole derivatives are key structural features commonly found in biologically active natural products[2,3] as tryptophan,[4] tryptamine[5] and auxin.[6] It has been reported that sharing of the indole 3-carbon in the formation of spiroindoline derivatives profoundly enhances the biological activity of the formed compounds.[7] Moreover, some of the compounds containing benzenesulfonamide moiety also show a broad spectrum of important biological properties such as elastase inhibition,[8] carbonic anhydrase inhibition,[9] *Clostridium histolyticum* collagenase inhibition[10] as well as herbicides and plant growth regulator effect.[11]

Sulfonamides are common motifs in many drugs and medicinal compounds and play an essential role in their bioactivity.[12] Common drugs such as glibenclamide,[13] sultiame,[14] and COX-II inhibitors like Piroxicam,[15] Ampiroxicam,[16] and Celecoxib[17] containing a sulfonyl moiety. The sulfonamides have attracted increasing attention for their excellent biological activity[18-20] including antitumor, antibacterial, thrombin inhibition and antifungal activities.[21-23]

In continuation of our previous work on triazoles, pyrimidine, thiazoles and thiazolidinones of pharmaceutical interest[24,25] we report here the synthesis and anticancer activity of several new 3-(indoline-1-carbonyl)-N-(substituted)benzenesulfonamide derivatives.

RESULTS AND DISCUSSION

We have synthesized new indole derivatives containing sulfonamide linkage. The synthetic methods for the preparation of the N-(substituted phenyl)-3-(indoline-1-carbonyl)benzenesulfonamide derivatives **(5a-g)** are presented at Scheme 1. We have screened peptide coupling conditions in (Table 1) to obtain better yield, excellent purity, shorter reaction time, avoiding costly reagents and mainly reproducibility of yields



Scheme 1 Synthesis of N-(substituted phenyl)-3-(indoline-1-carbonyl)benzenesulfonamides. **3a-5a**: R^1=2-methyl; **3b-5b**: R^1=2-ethyl; **3c-5c**: R^1=2-CF_3, **3d-5d**: 2-t-Bu; **3e-5e**: R^1=indoline, **3f-5f**: R^1=2-Me, 4-t-Bu; **3g-5g**: R^1=H. **Reagents and conditions:** (a) sulfonyl chloride, dichloromethane (DCM) 0 ^{0}C-rt; (b) substituted amine, pyridine, DCM, 0 ^{0}C-rt; (c) lithium hydroxide (LiOH), tetrahydrofuran (THF), water (H_2O), rt; (d) Indoline, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), diisopropylethyl-amine (DIPEA), DCM, rt.

The synthesis of compound **2** was done by treatment of**1** with sulfonyl chloride at 0 ^{0}C in DCM for 60 min and room temperature for 1 h. The reaction mixture was evaporated under reduced pressure and the obtained gummy material was washed with an excess of n-hexane. Recrystallization using 20 % ethyl acetate: n-hexane mixture gave pure **2** as yellow solid

Table 1. Optimization of peptide coupling reactions (**5a-g**)

Coupling Reagent	Base	Solvent	Time, h	Yield, %
HATU (1.1 equiv)	TEA (1.2 equiv)	DMF	14	57
HATU (1.1 equiv)	DIPEA (1.2 equiv)	DMF	14	55
PyBOP (1.1 equiv)	TEA (1.2 equiv)	THF	14	45
PyBOP (1.1 equiv)	DIPEA (1.2 equiv)	THF	14	50
EDCI (1.5 equiv)	TEA (2.5 equiv.)	DMF	14	62
HOBt (1.5 equiv)				
EDCI (1.5 equiv)	DIPEA (2.5 equiv)	DMF	14	72
HOBt (1.5 equiv)				
EDCI (1.5 equiv)	TEA (4 equiv)	DMF	14	78
HOBt (1.5 equiv)	DIPEA (4 equiv)	DMF	14	67
T3P (1.2 equiv)	TEA (2.5 equiv)	DCM	10	50
T3P (1.2 equiv)	DIPEA (2.5equiv)	DCM	10	60
EDCI (1.5 equiv)	DIPEA (2.5 equiv)	DCM	10	95
Acid (1 equiv) and Indoline (1.2 equiv)				

PyBOP=benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate;TEA=triethylamine; HATU=1-[bis(dimethylamino)-methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate; EDCI=1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOBt=hydroxybenzotriazole, T3P=propylphosphonic anhydride; DIPEA=diisopropylethylamine; DMF-dimethyformamid; DCM-dichloromethane; THF-tetrahydrofuran

Table 2. In vitro anticancer screening of the synthesized compounds **4a-4g** and **5a-5g** against A549, HeLa, MCF-7 and Du-145 cell lines. Data are expressed as IC_{50} (µM) ± SD (*n* = 3)

Compound	A549	HeLa	MCF-7	Du-145
4a	1.98±0.12	3.83±0.16	3.52±0.06	3.86±0.16
4b	2.81±0.13	2.92±0.08	2.32±0.22	3.82±0.12
4c	4.81±0.12	6.32±0.04	4.32±0.06	3.73±0.12
4d	2.82±0.11	1.99±0.22	2.36±0.12	3.52±0.11
4e	3.86±0.08	4.38±0.06	3.63±0.12	6.52±0.22
4f	2.72±0.11	3.87±0.08	4.12±0.06	3.86±0.22
4g	3.14±0.14	3.98±0.12	4.86±0.11	4.57±0.11
5a	8.48±0.14	9.12±0.08	7.82±0.08	9.12±0.06
5b	3.82±0.08	4.13±0.12	3.13±0.11	3.52±0.08
5c	4.13±0.12	5.16±0.08	6.12±0.12	4.52±0.11
5d	2.06±0.12	2.12±0.08	2.52±0.16	5.12±0.08
5e	2.52±0.11	3.52±0.11	4.48±0.08	4.08±0.11
5f	4.48±0.08	4.98±0.11	5.17±0.22	5.18±0.18
5g	2.73±0.08	2.12±0.12	2.12±0.08	2.12±0.04
5-Fluorouracil	1.61±0.12	1.72±0.18	1.81±0.10	1.89±0.12

The synthesis of compounds **3a-3g** was performed with sulfonamide coupling using variously substituted amines and compound **2** in the presence of pyridine as base and DCM as solvent at room temperature for 4 h. The reaction mass was treated with cold 2 M aq. HCl and the precipitated solids were washed with cold diethyl ether and pentane. The white solids **(3a-3g)** yield was varied between 85 and 95 %. The compounds **4a-4g** were prepared with hydrolysis of the compounds **3a-3g** using lithium hydroxide, tetrahydrofuran and water at room temperature for 10 h. Washing under basic conditions and acidifying led to the desired products as white solids with the required purity. The compounds **4a-4g** yield was varied between 80 and 85 %.

In order to synthesize compounds **5a-5g,** a series of coupling reagents and bases, and various solvents and reaction times were tested. We have varied the molar ratio of reagents and bases used to get better yield and purity in order to avoid column purifications (Table 1).

Using 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazo-lo[4,5-*b*]py-ridinium 3-oxid hexafluorophosphate (HATU) as coupling reagent and DMF as solvent and triethylamine (TEA) and diisopropylethylamine (DIPEA) as bases, after 14 h, the yield of the product was 57 and 55 %, respectively. Using benzotriazol-1-yl-oxytripyrrolidino-phosphonium hexafluorophosphate (PyBOP) as coupling reagent and THF solvent, and TEA and DIPEA as a base, the obtained yields were 45 and 50%, respectively. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and hydroxybenzotriazole (HOBt) as coupling reagents with DMF as a solvent, with 2.5 and 4 equiv. of TEA and DIPEA as bases for 14 h, gave 62, 78, and 72 and 67 % yields, respectively. Propylphosphonic anhydride (T3P) as coupling reagent with TEA and DIPEA as bases in DCM gave 50 and 60 % yield, respectively. When EDCI (1.5 equiv) was used together with DIPEA (2.5 equiv) in DCM, the yields were 95 %. Working up does not require column chromatography, no costly reagents are required.

The IC_{50} values for **4a-4g** and **5a-5g** are presented in Table 2, where all compounds exhibit moderate to a good activity toward cancer cell lines compared to 5-fluorouracil as a positive control. In the case of the human lung cancer cell line (A549) compounds **4a, 4b, 4d, 4f, 5d** and **5g** were the most potent with IC_{50} values ranging from 1.98-2.82 µM. The **4b, 4d, 5d** and **5g** compounds showed activity against HeLa cell line (IC_{50} = 1.99-2.92 µM), while in case of the MCF-7 breast cancer cell line, the most potent compounds were **4d, 5d** and **5g** withIC_{50}values of 2.12-2.52 µM. Lower activity was observed for the synthesized compounds on the Du-145 prostate cancer cell line, where the most potent candidate was the compounds **5g** with an IC_{50} value of 2.12 µM.

Generally, the lung (A549) and cervical (HeLa) cancer cell lines were the most sensitive toward the synthesized compounds. Regarding broad-spectrum anticancer activity reveals that compounds **4b, 4d** and **5g** were the most active, showing effectivity toward all four cell lines. The structure-activity relationship (SAR) showed that less hindered substitutions like methyl and ethyl groups at ortho and para position of aromatic rings increase the anticancer activity at all four cell lines, while ortho trifluoromethyl and indole groups decrease the anticancer activity. Despite steric hindrances, **4b**, **4d, 5d** and **5g** show promising anticancer activity due to electron donating substituents, and generally, the compounds with electron donating groups on the aromatic ring have more considerable anticancer activity than the compounds with electron withdrawing groups.

EXPERIMENTAL PART

Sulfonyl chloride and various solvents were commercially available. The chemicals were purchased from Sigma-Aldrich and Avra labs. Reaction courses were monitored by TLC on silica gel precoated F254 Merck plates. Developed plates were examined with UV lamps (254 nm). IR spectra were recorded on an FT-IR (Bruker). Melting points were recorded on SRS Optimelt, melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Varian NMR spectrometer with DMSO-d_6 solvent. The chemical shifts are reported as δ ppm units (TMS). The following abbreviations are used; singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). mass spectra were taken with Micromass-Quattro-II of water mass spectrometer.

General experimental procedure for the synthesis of N-(substituted phenyl)-3-(indoline-1-carbonyl)benzenesulfonamides (5a-5g)

Preparation of ethyl 3-(chlorosulfonyl)benzoate (2)

To a stirred solution of ethyl benzoate (10 g, 67 mmol) in DCM (25 mL) cooled to 0 ^{0}C, chlorosulfonic acid (9 g, 73 mmol) was added dropwise and the mixture was stirred for 1h at the same temperature followed by stirring at room temperature for further 1 h. After completion of reaction, the reaction mixture was evaporated under reduced pressure and the obtained gummy material was washed with excess of hexane and crystallized from 20 % ethyl acetate:hexane mixture to obtain ethyl 3-(chlorosulfonyl)benzoate **(2)** as white solid which is used further for sulfonamide coupling reaction, Yield 54 g (81 %).

Preparation of ethyl 3-(N-(o-tolyl)sulfamoyl)benzoate (3a)

To a stirred solution of ethyl 3-(chlorosulfonyl)benzoate **(2)** (3 g, 10.1 mmol) in DCM (5 ml), pyridine (5 ml) was added and the mixture was stirred at room temperature for 10 min. The reaction mixture was cooled to 0 ºC and 2-methylaniline (1.6 g, 15.16 mmol) was added dropwise followed by stirring at room temperature for 3 h. The reaction was monitored by TLC and LCMS, after completion of the reaction the reaction mixture was poured into cold 2 M aqueous HCl (10 ml) and stirred the mixture for 30 min. The obtained solid was filtered and washed with an excess of water, cold diethyl ether (10 ml) and cold pentane (10 ml). Ethyl 3-(N-(o-tolyl)sulfamoyl)benzoate (**2)** was obtained as white solid. Yield 2.8 g (90 %).

Preparation of 3-(N-(o-tolyl)sulfamoyl)benzoic acid (4a)

To a stirred solution of ethyl 3-(N-(o-tolyl)sulfamoyl)benzoate (**3a)** (2 g, 5.40 mmol) in THF (10 ml), water (2 ml) and lithium hydroxide (0.377 g, 18.2 mmol) were added and the reaction mixture was stirred for 4 h. The progress of the reaction was monitored by TLC and LC-MS. After the completion of the reaction, the reaction mixture was evaporated under reduced pressure with obtaining a gummy material. After adding 10 ml of water, the mixture was extracted with diethyl ether (10 ml). The pH of the collected aqueous layer was adjusted to 4 by 6 M aq. HCl. A precipitate was formed, and the mixture was stirred for 30 min. The obtained solid was filtered off, washed it with an excess of water, cold diethyl ether (10 ml) and cold pentane (10 ml) to obtain the desired 3-(N-(o-tolyl)sulfamoyl)benzoic acid (**4a)** as white solids. Yield 1.6 g (90 %)

N-(substituted phenyl)-3-(indoline-1-carbonyl)benzenesulfonamide (5a)

The compound 3-(N-(o-tolyl)sulfamoyl)benzoic acid (**4a**) (0.2 g, 0.65 mmol) was treated with EDCI (0.188 g, 0.98 mmol) and DIPEA (0.34 ml, 1.96 mmol) in DCM (10 ml). Then 2,4-dimethylaniline (0.238 g, 1.96 mmol) was added and the mixture was stirred at room temperature for 4 h. The reaction was monitored with TLC. 10 ml of cold water was added and the mixture was stirred for 10 min, then extracted with 10 ml of DCM. The collected organic layer was washed with 1 M aqueous HCl and brine (10 ml). Evaporating the organic layer, the compound **5a** was obtained with 90 % purity**.** Purification was done by washing with 5:95 % of DCM:hexane mixture, when the solid obtained was further washed with cold diethyl ether (20 ml) and cold pentane (20 ml).

3-(Indoline-1-carbonyl)-N-(o-tolyl)benzenesulfonamide (5a)

White solid (0.242 g, 92 %). LC-MS m/z (%): 393 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H), 8.26 (s, 1H), 8.22 (d, J=7.6 Hz, 1H), 7.81 (d, J=8 Hz, 1H), 7.7 (d, J=8 Hz, 1H), 7.18-7.13 (m, 4H), 7.1 (d, J=7.6 Hz, 2H), 6.95-6.92 (m, 2H), 3.60 (t, 2H), 3.16 (t, 2H), 2.14 (s, 3H). HPLC-98.50 % RT-5.68 min. ^{13}C NMR ($CDCl_3$, 100 MHz): 17.65, 27.79,

51.54, 126.09, 126.38, 126.40, 126.43, 126.58, 129.23, 129.42, 130.82, 130.89, 131.38, 133.42, 133.62, 134.27, 134.65, 135.40, 135.41, 135.45, 141.09, 163.93.

N-(2-Ethylphenyl)-3-(indoline-1-carbonyl)benzenesulfonamide (5b)

White solid, (0.240 g, 92 %) LC-MS *m/z* (%): 406 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H), 8.27 (s, 1H), 8.22 (d, *J*=8 Hz, 1H), 7.84 (d, *J*=8 Hz, 1H), 7.71 (t, *J*=7.6 Hz, 1H), 7.21-7.13 (m, 4H), 7.08-7.01 (m, 4H), 3.63 (t, 2H), 3.08 (t, 2H), 2.16 (q, 2H), 0.96 (t, 3H). HPLC-99.53% RT-9.21 min. ^{13}C NMR ($CDCl_3$, 100 MHz): 14.39, 18.80, 27.57, 53.18, 111.2, 120.3, 122.3, 124.5, 126.15, 126.23, 126.62, 126.63, 126.66, 129.34, 129.46, 131.36, 133.44, 133.67, 133.88, 135.46, 140.52, 141.13, 164.0.

3-(Indoline-1-carbonyl)-N-(2-(trifluoromethyl)phenyl)benzenesulfonamide (5c)

White solid, (0.240 g, 94 %). LC-MS *m/z* (%): 446 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (s, 1H), 8.34 (s, 1H), 8.26 (d, *J*=7.2 Hz, 1H), 7.96 (d, *J*=7.6 Hz, 1H), 7.79 (d, 2H), 7.58 (t, *J*=8 Hz, 1H), 7.49 (t, *J*=7.8 Hz, 1H), 7.18 (d, *J*=8 Hz, 1H), 7.08-7.01 (m, 4H), 3.63 (t, 2H), 3.08 (t, 2H), HPLC-96.65%RT-4.89 min. ^{13}C NMR ($CDCl_3$, 100 MHz): 27.74, 52.51, 111.55, 115.07, 119.55, 125.6, 127.08, 127.09, 127.54, 128.58, 128.59, 129.23, 129.57, 130.86, 131.51, 133.29, 133.39, 133.59, 133.88, 135.36, 139.51,164.87.

N-(2-(tert-butyl)phenyl)-3-(indoline-1-carbonyl)benzenesulfonamide (5d)

White solid (0.240 g, 92 %), LC-MS *m/z* (%): 434 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (s, 1H), 8.32 (s, 1H), 8.24 (d, *J*=7.2 Hz, 1H), 7.94 (d, *J*=7.6 Hz, 1H), 7.77 (d, 2H), 7.54 (t, *J*=8 Hz, 1H), 7.45 (t, *J*=7.8 Hz, 1H), 7.16 (d, *J*=8 Hz, 1H), 7.06-7.02 (m, 4H), 3.61 (t, 2H), 3.06 (t, 2H), 1.43 (s, 9H). HPLC-99.33% RT-6.18 min. ^{13}C NMR ($CDCl_3$, 100 MHz): 27.65, 31.3, 53.79, 51.54, 126.09, 126.38, 126.40, 126.43, 126.58, 129.23, 129.42, 130.82, 130.89, 131.38, 133.42, 133.62, 134.27, 134.65, 135.40, 135.41, 135.45, 141.09, 163.93.

Indolin-1-yl(3-(indolin-1-ylsulfonyl)phenyl)methanone (5e)

White solid (0.244 g, 94 %). LC-MS m/z (%): 404 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (s, 1H), 8.22 (d, J=7.6 Hz, 1H), 7.81 (d, J=8 Hz, 1H), 7.7 (t, J=8 Hz, 1H), 7.20-7.15 (m, 4H), 7.06-7.02 (m, 4H), 4.11 (t, 2H), 3.65 (t, 2H), 3.11 (t, 2H), 3.06 (t, 2H). HPLC-97.99 %. RT-8.42 min. ^{13}C NMR ($CDCl_3$, 100 MHz): 26.65, 27.79, 42.40, 51.54, 111.23, 126.09, 126.38, 126.40, 126.43, 126.58, 129.23, 129.42, 130.82, 130.89, 131.38, 133.42, 134.27, 134.65, 135.40, 135.41, 135.45, 141.09, 163.93.

N-(4-(tert-butyl)-2-methylphenyl)-3-(indoline-1-carbonyl)-benzenesulfonamide (5f)

White solid (0.238 g, 90 %), LC-MS *m/z* (%): 448 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.62 (s, 1H), 8.27 (s, 1H), 8.23 (d, *J*=7.2 Hz, 1H), 7.87 (d, *J*=7.6 Hz, 1H), 7.73 (d, *J*=7,6 Hz, 1H), 7.29 (d, *J*=8.4 Hz, 2H), 7.06-7.02 (m, 4H), 6.83 (d, *J*=8.4 Hz, 1H), 3.61 (t, 2H), 3.05 (t, 2H), 2.04 (s, 3H), 1.21 (s, 9H). HPLC-98.67% RT-7.43 min. ^{13}C NMR ($CDCl_3$, 100 MHz): 27.65, 31.79, 34.21, 52.33, 111.56, 120.19, 123.17, 125.17, 126.03, 126.29, 126.32, 126.34, 127.64, 129.3, 129.49, 131.24, 131.95, 133.85, 135.35, 139.56, 141.42, 148.88, 151.78, 163.81.

3-(Indoline-1-carbonyl)-N-phenylbenzenesulfonamide (5g)

(0.244 g, 94 %) as White solid, LC-MS *m/z* (%): 378 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 8.28 (s, 1H), 8.24 (d, *J*=7.6 Hz, 1H), 7.85 (d, *J*=8 Hz, 1H), 7.70 (d, *J*=8 Hz, 1H), 7.16-7.13 (m, 5H), 7.06-7.02 (m, 4H), 3.61 (t, 2H), 3.05 (t, 2H). HPLC-93.70% RT-7.58 min.^{13}C NMR ($CDCl_3$, 100 MHz): 27.34, 53.63, 119.40, 122.34, 125.63, 126.03, 126.12, 126.29, 126.71, 126.84, 128.2, 129.01, 129.37, 129.53, 129.94, 130.31, 133.87, 135.11, 136.24, 140.41, 165.03.

Anticancer activity

The synthesized compounds were evaluated for their *in vitro* anticancer activity against human lung cancer cell line (A549), cervical (HeLa) cancer cell line, breast cancer cell line (MCF-7) and prostate cell line (DU-145) using 5-fluorouracil as reference drug.[26] The IC_{50} value which corresponds to the concentration required for 50% inhibition of cell viability was determined.

Briefly, cells are grown in 96‐well plates in suspension and then were exposed for 48 hours to four serial concentrations of 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 1×10^{-4} and 1×10^{-3} M of each compound. Following this, cells were fixed and stained with protein binding SRB stain. Excess stain is washed out and the bound stain was solubilized, and the absorbance was measured at 492 nm in a plate reader. The concentration of the compounds that inhibited 50 % of the net cell growth was calculated from the dose-response curve obtained for each test compound and cell line. IC_{50} values were presented in micromolar (μM) concentration. 5‐Fluorouracil (5‐Fu) was used as positive control for the comparison of cytotoxicity of synthesized compounds. Assays were performed in triplicate on three independent experiments and their mean

CONCLUSION

An effective method was developed which provides easy access to new N-(substituted phenyl)-3-(indoline-1-carbonyl)benzenesulfonamide (**5a-g**) analogs. The mild reaction conditions, good to excellent yields, easiness of workup and the available substrates make the reactions to be attractive for the preparation of this compound class. The compounds (**4b, 4d, 5d** and**5g**) show potent anticancer activity in all the four cell lines tested.

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NEW THIAZOLONE DERIVATIVES: DESIGN, SYNTHESIS, ANTICANCER AND ANTIMICROBIAL ACTIVITY

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In order to explore the anticancer and antimicrobial activity associated with the thiazolone framework, several new (Z)-2-((5-(3-fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)carboxylic acid derivatives have been synthesized in water as a solvent. All synthesized compounds were evaluated for anticancer and antimicrobial activity in vitro. Amongst these, the 3-methylbutanoic and the 3- or 4-methylpentanoic acid derivatives, the 3-hydroxy-, the 3-(1H-imidazol-4-yl) and the 3-(4-hydroxyphenyl)propanoic acid derivatives and the succinic acid derivative showed high antibacterial and antifungal activity. The unsubstituted propanoic acid derivative exhibited significant antibacterial activity against *B. subtilis* and significant antifungal activity against fungal strains, i.e., *A. flavus*. The in vitro anticancer studies revealed that the 3-(hydroxy)-, the 3-(1H-imidazol-4yl)- and the 3-(4-hydroxyphenyl)propanoic acid, or the succinic acid derivatives are the most active compounds against MCF-7 and BT-474 human breast cancer cell lines.

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INTRODUCTION

Since the last five decade very rapid progress has been made in the area of cancer cell biology, though most cancer treatments are still multimodal, involving the chemotherapy.[1] Cancer is the second leading cause of death in the world after cardiovascular diseases and it is projected to begin the primary cause of death there within the coming year.[2,3] The breast cancer may be one of the oldest known forms of cancerous tumors in humans. Worldwide, breast cancer is the most common cancer in women, after skin cancer, representing 16 % of all female cancers.[4]

The heterocyclic chemistry has great importance for the medicinal chemists due to the high therapeutic activity of heterocyclic compounds. The compounds containing the 2-thioxothiazolidin-4-one (rhodanine) ring scaffold has been gaining prominence in recent years, because its derivatives are known to possess a broad spectrum of pharmacological activities, such as antimicrobial,[5-9] antidiabetic,[10] anticancer,[11-14] antiviral,[15,16] antifungal,[17] anticonvulsant,[18] anti-tuberculosis[19,20] and anti-HIV.[21,22] The identification of new structures that can be potentially useful in designing new, potent selective and less toxic anticancer agent is still a significant challenge to medicinal chemistry researchers.[23,24] The recent reports suggested that a chain containing free carboxyl group at the rhodanine nucleus had importance in the observed anticancer and antimicrobial activity.[25,26]

We initiated a program to synthesize thiazolone derivatives having amino acids chain as antimicrobial agents. In continuation of our work,[27] we have developed the new protocol for the synthesis of (Z)-5-(3-fluorobenzylidene)-2-thioxothiazolidin-4-one (**3**) (Scheme 1) by the condensation of 2-thioxothiazolidin-4-one and 3-fluorobenzaldehyde. The compound (**3**) was then subjected to a Knoevenagel condensation with the appropriate rhodamines synthesized using the reported procedure.[28,29]

EXPERIMENTAL SECTION

The compounds 2-thioxothiazolidin-4-one, 3-fluorobenzaldehyde, anhydrous sodium acetate, triethylamine, amino acids, dichloromethane, iodomethane and various solvents were commercially available (Sigma-Aldrich and Avra labs). Reaction courses were monitored by TLC on silica gel precoated F254 Merck plates. Developed plates were examined with UV lamps (254 nm). IR spectra were recorded on an FT-IR (Bruker). Melting points were recorded on SRS Optimelt, melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a 400 MHz Bruker spectrometer and were recorded in DMSO-d_6 solvent ^{13}C NMR spectra were recorded in DMSO-d_6 solvent on a 100 MHz Bruker spectrometer. Chemical shifts are reported as δ ppm units (TMS). The following abbreviations are used; singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer.

The general procedure of (Z)-5-(3-fluorobenzylidene)-2-thioxothia-zolidin-4-one (3)

In a 100 ml round bottom flask, the equimolar amount of the 2-thioxothiazolidin-4-one (1 mmol) and anhydrous sodium acetate (1 mmol) were mixed in glacial acetic acid (1 ml) with 3-fluorobenzaldehyde. The reaction mixture was stirred under reflux condition for 4 h. The progress of the reaction was monitored by TLC (20 % ethyl acetate: n-

hexane). After completion of the reaction, the reaction mixture was poured into the ice-cold water. The precipitate was filtered, washed with water (3×10 mL), dried, and purified by recrystallization from ethanol as a solvent to give a 90 % yield.

Yellow solid. Yield: 90 %. m.p. 198–200 ºC; ES-MS m/z (%): 239.29, IR ν_{max}/cm^{-1}: 3010 (NH), 1702 (C=O), 1594 (C=C), 1483 (C=N), 1436 (C=S), 1279 (C–N). ^{1}H NMR (DMSO-d_6): δ = 7.30–7.32 (m, 4H, Ar–CH), 7.60 (s, 1H, =CH), 13.90 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ = 113.5, 114.5, 116.3, 124.7, 130.9, 136.4, 143.2, 163.5, 168.4, 193.7.

General procedure of (Z)-5-(3-fluorobenzylidene)-2-(methylthio)thiazol-4(5H)-one (4)

In a 100 ml round bottom flask, the compound (**3**) (1 mmol) and triethylamine (1.5 mmol) were added in dichloromethane (10 ml) at room temperature. Iodomethane (1.5 mmol) was added to the stirred reaction mixture and the mixture was stirred for 2 h at room temperature. The progress of the reaction was monitored by TLC (10 % chloroform: methanol). After completion of the reaction, the reaction mixture was concentrated in-vacuo. The residue was washed out with water (3×15 mL) to afford the crude product. The crude product was recrystallized using ethanol as solvent.

Yellow solid. Yield: 92 %. m.p. 160–162 ºC; ES-MS m/z (%): 253.05, IR ν_{max}/cm^{-1}: 1703 (C=O), 1599 (C=C), 1577 (C=N), 1287 (C=S), 1086 (C–N). ^{1}H NMR (DMSO-d_6): δ = 2.90 (s, 3H, S-CH3), 7.10–7.21 (m, 4H, Ar–CH), 7.82 (s, 1H, =CH), ^{13}C NMR (DMSO-d_6): δ = 14.5, 113.9, 114.5, 124.1, 130.9, 136.4, 143.2, 152.0, 162.8, 163.5, 167.4.

General procedure of (Z)-2-((5-(3-fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) substituted carboxylic acids (6a-l).

In a 100 ml round bottom flask, the compound (**4**) (1 mmol), amino acids (**5a-l**) (1.5 mmol) and potassium carbonate (1.5 mmol) were mixed in water (1 mL) at room temperature and the reaction mixture was stirred for 30-55 min at room temperature. The progress of reaction was monitored by TLC (10 % chloroform: methanol). After completion of reaction, the reaction mixture was concentrated in-vacuo. The residue was washed with water (3×15 mL) to afford the crude product. The compounds (**6a-l**) were recrystallized from ethanol and isolated as yellowish solids.

(Z)-2-((5-(3-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)propanoic acid (6a)

Yellow solid. Yield: 92 %, m.p. 189–191 ºC; ES-MS m/z (%): 294.28, IR ν_{max}/cm^{-1}: 3744 (COOH), 3397 (OH), 1742 (HO–C=O), 1642 (C=O), 1564 (C=C), 1549 (C=N), 1206 (C-S), 1086 (C–N). ^{1}H NMR (DMSO-d_6): δ = 1.40–1.42 (d, 3H, C–CH3), 4.55–4.57 (q, 1H, CH), 7.30–7.32 (m, 4H, Ar–CH), 7.70 (s, 1H, =CH), 10.20 (s, 1H, NH), 12.65 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6): δ=16.8, 53.5, 113.9, 114.5, 124.2, 129.7, 132.7, 136.5, 152.1, 158.3, 162.3, 167.7, 174.2.

(Z)-2-((5-(3-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-3-methylbutanoic acid (6b)

Yellow solid. Yield: 94 %, m.p. 204–206 ºC; ES-MS m/z (%): 322.35, IR ν_{max}/cm^{-1}: 3414 (OH), 3215 (NH), 3013 (CH–Ar), 1732 (HO–C=O), 1684 (C=O), 1551 (C=C), 1593 (C=N), 1012 (C-S), 1091 (C–N). ^{1}H NMR (DMSO-d_6): δ = 0.91–0.93 (d, 6H, CH(CH3)2), 1.50–1.52 (m, 1H, CH), 4.41–4.43 (d, 1H, CH), 7.25–7.27 (m, 4H, Ar–CH), 7.88 (s, 1H, =CH), 11.62 (s, 1H, NH), 13.12 (s, 1H, COOH). 13C NMR (DMSO-d_6): δ = 18.8, 30.5, 61.3, 113.8, 114.4, 124.2, 130.2, 132.3, 135.9, 152.3, 158.1, 162.7, 167.2, 174.2.

(Z)-2-((5-(3-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-3-methylpentanoic acid (6c)

Yellow solid. Yield: 92 %, m.p. 165–167 ºC; ES-MS m/z (%): 336.38, IR ν_{max}/cm^{-1}: 3398 (OH), 3212 (NH), 3017 (CH–Ar), 1735 (HO–C=O), 1692 (C=O), 1557 (C=C), 1583 (C=N), 1013 (C-S), 1097 (C–N). ^{1}H NMR (DMSO-d_6): δ = 0.91–0.93 (t, 3H, CH_2–CH_3), 1.15–1.17 (d, 3H, CH3) 1.52–1.54 (m, 2H, CH_2), 1.81–1.83 (m, 1H, CH), 4.43–4.45 (d, 1H, CH), 7.21–7.23 (m, 4H, Ar–CH), 7.80 (s, 1H, =CH), 11.64 (s, 1H, NH), 13.29 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6): δ = 11.2, 15.4, 25.7, 36.4, 58.4, 113.4, 114.5, 124.9, 130.6, 132.3, 135.9, 152.9, 158.1, 162.4, 167.3, 175.2.

(Z)-2-((5-(3-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)-amino)-3-phenylpropanoic acid (6d)

Yellow solid. Yield: 90 %, mp 138–140 ºC; ES-MS m/z (%): 370.40, IR ν_{max}/cm^{-1}: 3396 (OH), 3211 (NH), 2992 (CH–Ar), 1737 (HO–C=O), 1692 (C=O), 1551 (C=C), 1581 (C=N), 1017 (C-S), 1098 (C–N). ^{1}H NMR (DMSO-d_6): δ = 2.51–2.53 (d, 2H, CH2), 4.45–4.47 (q, 1H, CH), 7.25–7.27 (m, 9H, Ar–CH), 7.89 (s, 1H, =CH), 11.75 (s, 1H, NH), 13.31 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6): δ = 36.4, 58.4, 113.5, 114.6, 124.9, 125.9, 125.7, 128.6, 128.9, 130.7, 132.3, 135.3, 136.9, 152.2, 158.5, 167.1, 175.2.

(Z)-2-((5-(3-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)-amino)-4-(methylthio)butanoic acid (6e)

Yellow solid. Yield: 92 %, mp 142–144 ºC; ES-MS m/z (%): 354.42, IR ν_{max}/cm^{-1}: 3396 (OH), 3210 (NH), 2980 (CH–Ar), 1732 (HO–C=O), 1698 (C=O), 1541 (C=C), 1586 (C=N), 1014 (C-S), 1091 (C–N). ^{1}H NMR (DMSO-d_6): δ = 2.01–2.03 (q, 2H, CH_2), 2.20 (s, 3H, CH_3), 2.61–2.63 (t, 2H, CH2), 4.43–4.45 (q, 1H, CH), 7.30–7.32 (m, 4H, Ar–CH), 7.78 (s, 1H, =CH), 11.61 (s, 1H, NH), 13.15 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6): δ = 15.3, 29.8, 30.5, 56.8, 113.5, 114.6, 124.9, 127.7, 128.6, 136.9, 152.2, 158.5, 162.4, 167.3, 174.7.

(Z)-2-((5-(3-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)-amino)-4-methylpentanoic acid (6f)

Yellow solid. Yield: 94 %, mp 187–189 ºC; ES-MS m/z (%): 336.38, IR ν_{max}/cm^{-1}: 3397 (OH), 3217 (NH), 3011 (CH–Ar), 1736 (HO–C=O), 1695 (C=O), 1555 (C=C), 1583 (C=N), 1013 (C-S), 1091 (C–N). ^{1}H NMR (DMSO-d6): δ = 0.92–0.94 (d, 6H, CH–(CH_3)$_2$), 1.41–1.43 (m, 1H, CH),

1.71–1.73 (t, 2H, CH2), 4.44–4.46 (q, 1H, CH), 7.29–7.31 (m, 4H, Ar–CH), 7.76 (s, 1H, =CH), 11.72 (s, 1H, NH), 13.35 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6): δ = 22.7, 24.4, 40.3, 55.4, 113.4, 114.4, 124.9, 129.2, 132.1, 135.4, 152.2, 159.1, 162.3, 167.1, 174.2.

(Z)-2-((5-(3-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)-amino)-3-hydroxypropanoic acid (6g)

Yellow solid. Yield: 96 %, m.p. 185–187 ºC; ES-MS m/z (%): 310.30, IR ν_{max}/cm^{-1}: 3450 (OH), 3211 (NH), 3008 (CH–Ar), 1738 (HO–C=O), 1688 (C=O), 1551 (C=C), 1511 (C=N), 1019 (C-S), 1097 (C–N). ^{1}H NMR (DMSO-d_6): δ = 3.62 (s, 1H, CH), 4.02–4.04 (t, 1H, CH), 4.23–4.25 (d, 2H, CH_2), 7.31–7.33 (m, 4H, Ar–CH), 7.93 (s, 1H, =CH), 11.60 (s, 1H, NH), 13.13 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6): δ = 59.2, 62.2, 113.4, 114.4, 124.9, 129.2, 130.3, 132.1, 136.4, 151.9, 158.1, 167.9, 172.2.

(Z)-2-((5-(3-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)-amino)-3-mercaptopropanoic acid (6h)

Yellow solid. Yield: 94 %, mp 179–181 ºC; ES-MS m/z (%): 326.70, IR ν_{max}/cm^{-1}: 3455 (OH), 3201 (NH), 3017 (CH–Ar), 2500 (SH), 1738 (HO–C=O), 1695 (C=O), 1559 (C=C), 1503 (C=N), 1014 (C-S), 1095 (C–N). ^{1}H NMR (DMSO-d_6): δ = 1.50 (s, 1H, SH), 3.11–3.13 (d, 2H, CH_2), 4.13–4.15 (t, 1H, CH), 7.22–7.24 (m, 4H, Ar–CH), 7.68 (s, 1H, =CH), 11.61 (s, 1H, NH), 13.11 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6): δ = 26.3, 60.4, 113.4, 114.4, 124.9, 127.5, 132.5, 135.6, 152.9, 158.3, 162.3, 167.2, 178.2.

(Z)-2-((5-(3-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)-amino)succinic acid (6i)

Yellow solid. Yield: 92 %, mp 214–216 ºC; ES-MS m/z (%): 338.40, IR ν_{max}/cm^{-1}: 3464 (OH), 3213 (NH), 3020 (CH–Ar), 1732 (HO–C=O), 1689 (C=O), 1549 (C=C), 1503 (C=N), 1030 (C-S), 1089 (C–N). ^{1}H NMR (DMSO-d_6): δ = 2.60–2.62 (d, 2H, CH_2), 3.71–3.73 (t, 1H, CH), 7.35–7.37 (m, 4H, Ar–CH), 7.88 (s, 1H, =CH), 11.70 (s, 1H, NH), 13.12 (s, 2H, COOH). ^{13}C NMR (DMSO-d_6): δ = 35.9, 53.3, 113.4, 114.4, 124.9, 130.1, 132.5, 135.6, 136.4, 152.9, 158.3, 162.3, 167.2, 178.2.

(Z)-2-((5-(3-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)-amino)-3-(1H-imidazol-4-yl)propanoic acid (6j)

Yellow solid. Yield: 94 %, m.p. 152–154 ºC; ES-MS m/z (%): 360.38, IR ν_{max}/cm^{-1}: 3435 (OH), 3215 (NH), 3011 (CH–Ar), 1739 (HO–C=O), 1681 (C=O), 1552 (C=C), 1508 (C=N), 1033 (C-S), 1092 (C–N). ^{1}H NMR (DMSO-d_6): δ = 2.92–3.94 (d, 2H, CH_2), 3.73–3.86 (t, 1H, CH), 7.31–7.33 (m, 4H, Ar–CH), 7.77 (s, 1H, =CH), 7.86 (s, 1H, =CH), 8.98 (s, 1H, =CH), 11.62 (s, 1H, NH), 13.12 (s, 1H, NH), 13.34 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6): δ = 28.9, 58.3, 113.4, 114.4, 117.9, 124.9, 124.7, 124.7, 127.9, 128.6, 132.1, 136.2, 152.3, 158.2, 167.5, 176.2.

(Z)-2-((5-(3-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-3-(4-hydroxyphenyl)propanoic acid (6k)

Yellow solid. Yield: 92 %, m.p. 212–214 ºC; ES-MS m/z (%): 386.44, IR ν_{max}/cm^{-1}: 3462 (O=C-OH), 3392 (OH), 3211 (NH), 2992 (CH–Ar), 1732 (HO–C=O), 1697 (C=O), 1553 (C=C), 1591 (C=N), 1011 (C-S), 1089 (C–N). ^{1}H NMR (DMSO-d_6): δ = 2.80–2.98 (d, 2H, CH2), 4.43–4.45 (t, 1H, CH), 5.30 (s, 1H, OH), 7.30–7.32 (m, 8H, Ar–CH), 7.87 (s, 1H, =CH), 11.72 (s, 1H, NH), 13.32 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6): δ = 36.3, 58.6, 113.9, 114.7, 115.8, 127.7, 128.9, 129.2, 130.2, 135.3, 136.9, 152.2, 155.7, 158.5, 162.7, 167.8, 174.3.

(Z)-2-((5-(3-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-3-hydroxybutanoic acid (6l)

Yellow solid. Yield: 90 %, m.p. 173–175 ºC; ES-MS m/z (%): 324.56, IR ν_{max}/cm^{-1}: 3462 (OH), 3201 (NH), 3010 (CH–Ar), 1733 (HO–C=O), 1682 (C=O), 1534 (C=C), 1516 (C=N), 1042 (C-S), 1115 (C–N). ^{1}H NMR (DMSO-d6): δ = 1.10–1.12 (d, 3H, CH_3), 3.51–3.55 (d, 1H, CH), 3.63 (s, 1H, OH), 3.93–3.95 (m, 1H, CH), 7.25–7.27 (m, 4H, Ar–CH), 7.75 (s, 1H, =CH), 11.60 (s, 1H, NH), 13.10 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6): δ = 19.6, 64.3, 66.5, 113.9, 114.7, 124.8, 130.3, 132.4, 136.1, 152.2, 158.3, 162.4, 167.8, 174.2.

Antimicrobial tests

The antimicrobial activity of compounds were tested against six bacteria; *Bacillus subtilis* (NCIM-2063), *Staphylococcus aureus* (NCIM-2901), *Escherichia coli* (NCIM-2256), *Enterococcus faecalis* (NCIM-5443), *Pseudomonas aeruginosa* (NCIM-2037), *Salmonella typhimurium* (NCIM-2501) and six fungal strains; *Aspergillus oryzae* (NCIM-570), *Penicillium chrysogenum* (NCIM-707), *Fusarium oxysporum* (NCIM-1282), *Candida albicans* (NCIM-3471), *Aspergillus flavus* (NCIM-539) and *Aspergillus niger* (NCIM-1196). The antibacterial activity of compounds was monitored by observing their Minimum inhibitory concentration (MIC, μg mL^{-1}) as previously mentioned[30] by broth dilution methods with Ciprofloxacin and Ampicillin as control drugs.

The antifungal study was carried by the standard agar dilution method and Fluconazole and Miconazole were used as control drugs. Ethanol was used as solvent control for both antibacterial and antifungal testing.

All the synthesized compounds were also tested for their general cytotoxicity on MCF-7 and BT-474 human breast cancer cell line. This test is performed as previously mentioned MTT colorimetric assay.[31] Cytotoxicity of the compounds was determined by calculating their IC_{50} values (μM mL^{-1}), the concentration of compound required to inhibit 50 % of cell growth compared to untreated control cells. The results are given as percentage cytotoxicity after 24 h. Adriamycin was used as positive control for the comparison of cytotoxicity of synthesized compounds. Assays were performed in triplicate on three independent experiments.

RESULT AND DISCUSSION

Several new (Z)-2-((5-(3-fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) substituted organic acid derivatives (**6a-l**) were synthesized and characterized by ^{1}H and ^{13}C NMR, IR spectroscopy.

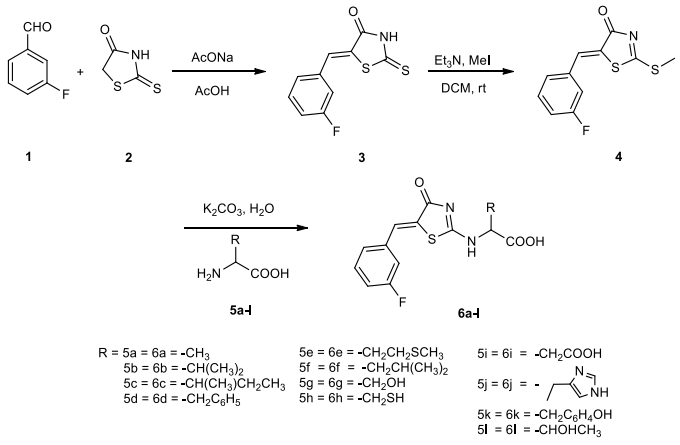
A new protocol for the synthesis of (Z)-5-(3-fluorobenzylidene)-2-thioxothiazolidin-4-one (**3**) by the condensation of 2-thioxothiazolidin-4-one and 3-fluorobenzaldehyde as the initial step of the reaction route has been developed.

Table 1. Physical data of the synthesized compounds **3**, **4** and **6a-l**[a]

Comp.	Substituent (R)	Time, min	Yield, %[b]	M.P., °C
3	-	240	90	198-200
4	-	120	92	160-162
6a	$-CH_3$	35	92	189-191
6b	$-CH(CH_3)_2$	30	94	204-206
6c	$-CH(CH_3)CH_2CH_3$	35	92	165-167
6d	$-CH_2C_6H_5$	35	90	138-140
6e	$-CH_2CH_2SCH_3$	40	92	142-144
6f	$-CH_2CH(CH_3)_2$	55	94	187-189
6g	$-CH_2OH$	50	96	185-187
6h	$-CH_2SH$	40	94	179-181
6i	$-CH_2COOH$	50	92	214-216
6j	3-(1H-imidazol-4yl)	35	94	152-154
6k	$-CH_2C_6H_4OH$	45	92	212-214
6l	$-CHOHCH_3$	35	90	173-175

[a]Reaction condition (**6a-l**): Compound (**4**) (1 mmol), amino acids (**5a-l**) (1.5 mmol), K_2CO_3 (1.5 mmol), 1 ml water at room temperature.[b]Isolated yields

The compound (**3**) was then subjected to a Knoevenagel condensation with the appropriate 2-thioxothiazolidin-4-one, to synthesize a new series of target compounds (**6a-l**).



Scheme 1 Synthesis of (Z)-2-((5-(3-fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) substituted acid (**6a-l**).

The structures of the desired compounds were confirmed by IR, ^{1}H NMR and ^{13}C NMR. The compound **3** was prepared in prominent good yields via a Knoevenagel condensation between the corresponding heterocyclic cores of Rhodanine and 3-fluorobenzaldehyde (Scheme 1).

The 2-thioxothiazolidin-4-one based compounds were synthesized by conventional heating with sodium acetate, which acts as a base and glacial acetic acid as catalysts. In theory, of E and Z geometrical isomers may exist according to the configuration around the exocyclic double bond (CH=C) for (Z)-5-(3-fluorobenzylidene)-2-thioxothiazolidin-4-one (**3**).

The ^{1}H NMR spectrum of the compound **3** shows only one signal for the methine proton in the range δ 7.66, which show the presence of one isomer only, and at lower field values than those expected for the E-isomers, which was strongly indicated that the compounds have the Z-configuration. The IR spectrum of compound **3**, showed a strong absorption band at 1702 cm^{-1} that is due to a carbonyl group. The mass spectrum revealed a molecular ion peak at m/z=239.29 corresponding to a molecular formula $C_{10}H_6FNOS_2$. The latter has been reported as thermodynamically more stable than the E-configuration.[32,33]

Compound **4** was synthesized from compound **3** and the structures of the desired product were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. The IR spectrum of (Z)-5-(3-fluorobenzylidene)-2-(methylthio)-thiazol-4(5H)-one (**4**), showed a strong absorption band at 1704 cm^{-1} belongs to a carbonyl group. The mass spectrum revealed a molecular ion peak at m/z = 253.05 corresponding to a molecular formula $C_{11}H_8FNOS_2$. The ^{1}H NMR spectra of the compound **4** show only one signal for the methine proton in the range δ 7.82, sulfur attached methyl group proton shows a singlet at δ 2.85.

We synthesized a series of new (Z)-2-((5-(3-fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) substituted carboxylic acids (**6a-l**) from the compound **4** and different types of amino acids (**5a-l**), (Scheme 1, Table 1). The displacement of a methylthio group by various amino acids from the C2 position of the thiazolone ring and the structures of the desired compounds (**6a-l**) were confirmed by IR, ^{1}H NMR, ^{13}C NMR analysis.

The IR spectrum of (Z)-2-((5-(3-fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)-amino)propanoic acid (**6a**), showed a strong absorption band at 1742 cm^{-1} belongs to the carbonyl of a carboxylic group and 3397 cm^{-1} due to the presence hydroxyl group. The mass spectrum revealed a molecular ion peak at m/z=294.28 corresponding to a molecular formula $C_{13}H_{11}FN_2O_3S$. The ^{1}H NMR spectrum revealed that the signals of **6a** (as a representative example), show the methyl group protons doublet in the range of δ 1.40–1.50, the methine proton (adjacent to carboxylic acid group) shows a quartet in the range of δ 4.50–4.70, phenyl ring protons show a multiplet in the range of δ 7.40–7.70, one signal appears for the alkene proton in the range δ 7.72, amine group proton shows singlet at δ 10.10, and the carboxylic acid proton shows singlet at δ 12.65.

Table 2. Antibacterial activity of synthesized compounds **3**, **4** and **6a-6l**

Compound		*B.s.*	*S.a.*	*E.c.*	*E.f.*	*P.a.*	*S.t.*
3	MIC	35.70	35.57	10.37	34.70	30.90	30.95
	MBC	55.13	56.15	36.40	46.14	33.76	34.75
4	MIC	31.70	31.50	31.39	35.13	35.30	33.90
	MBC	45.13	45.17	45.40	45.12	55.73	31.75
6a	MIC	36.14	36.12	33.17	34.16	28.35	28.35
	MBC	44.55	44.56	44.53	47.54	37.16	32.18
6b	MIC	7.10	13.10	48.40	48.10	52.13	52.15
	MBC	18.20	23.30	69..40	63.10	52.20	51.90
6c	MIC	55.70	56.57	56.37	33.70	33.90	6.85
	MBC	58.14	58.16	48.40	58.10	38.76	19.85
6d	MIC	33.70	33.50	32.39	35.10	25.30	37.90
	MBC	48.15	47.10	47.40	47.10	58.60	32.70
6e	MIC	9.50	8.75	30.10	34.10	28.50	27.30
	MBC	32.10	32.10	48.50	48.50	32.70	33.10
6f	MIC	7.45	8.78	48.40	48.10	52.13	52.15
	MBC	16.20	20.30	59..40	60.10	55.20	56.90
6g	MIC	38.70	38.57	19.37	38.70	32.90	6.95
	MBC	54.13	55.15	35.40	58.10	37.76	18.75
6h	MIC	33.70	33.50	33.39	35.10	26.30	36.90
	MBC	47.10	47.15	47.47	48.10	58.60	32.70
6i	MIC	9.50	8.10	30.10	34.10	28.50	27.30
	MBC	34.10	34.10	48.50	45.50	35.70	33.10
6j	MIC	7.10	8.50	38.40	38.10	42.13	51.15
	MBC	16.20	20.30	59..40	60.10	55.20	56.90
6k	MIC	38.70	38.57	18.37	39.70	33.90	8.95
	MBC	59.13	54.15	36.40	36.10	36.76	16.75
6l	MIC	37.10	38.10	39.40	36.10	32.13	32.15
	MBC	44.20	42.30	58..40	63.10	55.20	56.90
Ciprofloxacin	MIC	14.70	13.69	12.69	15.69	11.69	15.69
	MBC	33.19	33.10	22.10	32.10	14.10	34.10
Ampicillin	MIC	3.71	3.20	3.20	3.20	3.86	3.46
	MBC	5.29	5.43	1.43	1.43	2.71	1.86

[a]Values are the average of three readings. MIC= minimal inhibitory concentration, in mg L^{-1}, MBC = 1.43-5.43 in mg L^{-1}. B.s.- *Bacillus subtilis* (NCIM-2063), S.a.-*Staphylococcus aureus* (NCIM-2901), E.c.- *Escherichia coli* (NCIM-2256), E.f. - *Enterococcus faecalis* (NCIM-5443), P.a.- *Pseudomonas aeruginosa* (NCIM-2037), S.t.- *Salmonella typhimurium* (NCIM-2501)

The antimicrobial activities of the synthesized compounds against selected Gram-positive and Gram-negative bacteria and multidrug-resistant bacteria are illustrated in Table 2 and Table 3. The majority of the synthesized compounds show a variety of antibacterial, antifungal and cytotoxic activity. The compounds **3** was found to be the most active against *E. coli* and the compounds **6c**, **6g** and **6k** were found to be active against *S. typhimurium*.

Some of the studied compounds are more potent against selected microorganisms than a standard antibacterial drug Ciprofloxacin. For example, against *E. Coli* the compound **3** while against *B. subtilis* the compounds **6b**, **6e**, **6f**, and **6j** have better MIC values than the values found for Ciprofloxacin. Against *S. aureus*, the compounds **6b**, **6e**, **6f,6i** and **6j** proved to be more active than Ciprofloxacin.

The compounds **6g** and **6k** showed antifungal activity against four fungus strains, namely *A. oryzae*, *P. chrysogenum*, *C. albicans* and *A. flavus*. The compound **6a** showed activity against *A. flavus*, the compound **6c** against *P. Chrysogenum* and *F. oxysporum* while the compound **6e** was found to be active against *P. chrysogenum*. Remaining compounds of the series (**3**, **4**, **6d**, **6f**, **6h**, **6i**, **6j** and **6l** were found to be inactive against fungi.

All the synthesized compounds were tested for their cytotoxic activity against MCF-7 and BT-474 cell lines (Table 4). (Table 4). Three compounds, **6g**, **6i** and **6l** showed good activity against the studied cancer cell lines, the IC_{50} values against MFC-7 and BT-474 cell lines were found to be 1.4 and 1.3, 1.5 and 0.6, or 1.1 and 1.4 μM mL^{-1}, respectively. The IC_{50} values for reference drug Adriamycin against MFC-7 and BT-474 cells were found to be 0.9 and 0.5 μM mL^{-1}, respectively. The cytotoxicity of the newly synthesized thiazolones depends on the type of substituents on thiazolone moiety. The compounds containing hydroxyl groups attached to the amino acid parts linked to the thiazolone ring have the highest cytotoxic activity. The presence of electron releasing alkyl chain, methyl-1H-imidazole ring or thiol group on the amino acid attached to the thiazolone rings resulted in the loss of activity.

Table 3. Antifungal activity of synthesizing compounds **3**, **4** and **6a-l**

Compound		*A.o.*	*P.c.*	*F.o.*	*C.a.*	*A.f.*	*A.n.*
3	MIC	32.50	52.39	34.50	34.50	18.30	18.30
	MFC	53.10	53.10	74.12	54.10	35.20	75.33
4	MIC	37.00	37.20	33.20	33.20	33.20	30.00
	MFC	36.20	46.60	81.10	42.30	34.88	35.00
6a	MIC	31.50	31.39	31.50	34.50	12.30	19.30
	MFC	46.10	46.10	62.12	32.10	33.20	41.33
6b	MIC	33.00	31.20	31.20	32.20	32.20	34.00
	MFC	43.20	44.50	45.10	66.30	33.88	48.00
6c	MIC	35.50	17.39	17.50	57.50	36.30	39.30
	MFC	49.40	34.40	34.32	36.30	34.30	35.33
6d	MIC	32.30	35.40	32.30	57.50	67.50	34.50
	MFC	42.30	52.20	84.10	52.00	72.30	51.50
6e	MIC	36.30	13.80	51.20	31.25	44.25	30.55
	MFC	36.80	34.70	65.18	60.18	30.64	45.69
6f	MIC	34.00	34.20	34.20	55.20	34.70	31.00
	MFC	43.20	44.60	49.10	65.35	35.88	48.00
6g	MIC	5.55	16.36	39.50	18.50	16.30	36.30
	MFC	10.10	44.10	32.11	35.10	32.22	32.23
6h	MIC	32.00	32.50	34.40	68.40	58.10	34.40
	MFC	51.34	52.25	84.15	72.05	33.30	54.50
6i	MIC	42.35	55.80	41.26	31.26	74.26	35.58
	MFC	55.80	35.78	62.38	62.38	32.84	43.29
6j	MIC	43.40	34.24	54.26	34.66	34.26	33.07
	MFC	53.25	42.65	43.15	64.48	33.89	58.08
6k	MIC	34.55	17.30	16.50	16.50	16.30	33.30
	MFC	45.10	33.75	53.52	32.10	34.20	35.33
6l	MIC	42.00	32.60	62.50	59.50	58.70	34.00
	MFC	58.30	55.20	64.10	62.50	62.30	51.50
Fluconazole	MIC	5.60	1.68	28.65	5.70	9.42	2.28
	MFC	9.35	5.75	46.00	9.62	17.80	5.75
Miconazole	MIC	40.25	5.30	7.18	1.34	43.20	156.30
	MFC	85.18	151.28	20.20	6.18	142.20	140.12

[a]Values are the average of three readings. A.o.- Aspergillus oryzae (NCIM-570), P.c.- Penicillium chrysogenum (NCIM-707), F.o.- Fusarium oxysporum (NCIM-1282), C.a.- Candida albicans (NCIM-3471), A.f.- Aspergillus flavus (NCIM-539) and A.n.- Aspergillus Niger (NCIM-1196).

Structure-activity relationship (SAR)

The results of the antimicrobial screening demonstrated some facts about the structural-activity relationship (SAR) of the synthesized thiazolone derivatives. The notable highlights of structure-activity relationship are the followings:

The biological activity profile of molecules is strongly affected by the branching pattern and chain length of alkyl moieties. Attachment of a methyl group at C2 position on the thiazolone moiety (**6a**) makes molecule active against bacterial and fungal strains, probably due to its small size and electron donating effects. When this methyl group is replaced by isopropyl (**6b**) and 2-methylbutyl (**6c**) groups, the molecules become active on a broader spectrum (against the majority of the studied strains). This shows that the presence of branching at the carbon located on the C2 position of the thiazolone ring has a positive effect on appearing or strengthening of antimicrobial activity. Attachment of 2-methylpropyl group (**6f**) at the same position makes the molecule to be specific towards the *B. subtilis* and ***S. aureus***. Substitution of the alkane chain with a carboxylic group (**6i**), also resulted in specificity towards *B. subtilis* and *S. aureus.*

Table 4. In vitro cytotoxicity of compounds towards the MCF-7 and BT-474 cells, after 24 h.

Sr. No.	Compounds	IC_{50},[a] µM[b]	
		MCF-7[c]	BT-474[d]
1	**3**	38.6	53.4
2	**4**	54.8	25.0
3	**6a**	44.9	43.4
4	**6b**	46.2	62.2
5	**6c**	56.3	56.6
6	**6d**	82.4	48.6
7	**6e**	76.5	48.6
8	**6f**	82.4	56.8
9	**6g**	1.4	0.6
10	**6h**	72.2	77.1
11	**6i**	1.3	1.1
12	**6j**	78.7	74.5
13	**6k**	10.1	10.0
14	**6l**	1.5	1.4
	Adriamycin	0.9	0.5

[a]GI_{50}(Growth inhibition of 50 %): Concentration of drug that decreases the growth of the cells by 50 % compared to a non-treated control cell. [b]Values are the average of three readings; [c]MCF-7: Human Breast cancer cell line, [d]BT-474: Human Breast cancer cell line; e-Adriamycin: positive control compound

Substitution by phenylmethyl group (**6d**) at the C2 position of the thiazolone moiety gave completely inactive molecule towards all tested strains, while the 4-hydroxyphenyl (**6k**) substitution made the molecule to be active towards *S. typhimurium*, and *P. chrysogenum, F. oxysporum, C. Albicans* and *A. flavus*. The compound (**6j**) containing methyl-imidazole ring at the C2 position of the thiazolone moiety resulted in appearing of specific activity towards the Gram-positive bacteria *B. subtilis* and *S. aureus*. The compound (**6e**) with the terminal methylthio group is active toward.*B. subtilis, S. aureus* and *P. chrysogenum*, while compound (**6h**) with terminal mercapto group was proved to be inactive towards these bacterial and fungal strains.

CONCLUSION

The objective of our present study is to synthesize and investigation of the potent anticancer and antimicrobial activities of some new (Z)-2-((5-(3-fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) substituted organic acids.

This is the first reported synthesis of the (Z)-2-((5-(3-fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) substituted organic acids in water as solvent with excellent yields in shorter reaction time making the process economically lucrative for industrial application. Some derivatives were found to be more active against several bacteria and fungi strains than the common antimicrobial agents.

In vitro anticancer studies revealed that the compounds **6g**, **6i**, **6k** and **6l** are most active against MCF-7 and BT-474 human breast cancer cell lines.

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HYPOGLYCAEMIC ACTIVITY OF *VERNONIA AMYGDALINA* (DEL.) EXTRACTS IN NORMAL AND ALLOXAN-INDUCED RATS

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Keywords: Normo-glycaemic; alloxan; extracts; diabetes mellitus; hypoglycaemic.

Vernonia amygdalina (Del.) is widely cultivated in tropical Africa and known for its bitter principles. The extracts of this plant are used in folklore medicine to treat fevers, measles, tuberculosis, toothaches, parasitic infections, asthma, diarrhoea and in managing diabetes mellitus. The growing concerns arising from the treatment and management of the diabetes prompted this study. Hence, the hypoglycaemic potentials of leaf, stem and root (squeezed and methanolic) extracts of the plant were investigated in normo-glycaemic and alloxan-induced rats. The blood glucose levels in normal and diabetic rats were determined after the administration of 300mg/kg of extract and 150mg/kg of alloxan monohydrate at time (t) = 0, 1, 2 and 4 h. The hypoglycaemic activities of the squeezed extracts of leaves, stem and roots were not significant in both normal and diabetic rats. However, the methanolic extracts of the leaves and roots demonstrated significantly remarkable hypoglycaemic activities compared with the activity given by the stem extract. The methanolic extracts of leaves and root have shown to be effective in lowering blood glucose level (80% reduction after 4h) while the stem afforded a poorly 20% reduction after 4 h. The results from this study have lent scientific credence to the ethnobotanical use of the plant in the treatment and management of diabetes mellitus. However, the claims that the stem is as effective in the herbal therapy of this metabolic disease can not be supported by the results obtained from this study.

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INTRODUCTION

Medicinal plants are undoubtedly relevant in both developing and developed nations of the world as sources of herbal drugs for treating and managing various ailments and disease conditions. Herbal medicines are finished, labelled medicinal products which contain plant parts whether in the crude state or slightly processed state.[1] Hence, some plants are used for treating such high priority diseases such as cardiovascular conditions, sickle cell anaemia, HIV/AIDS, renal ailments, tumours and most especially diabetes mellitus.[2] Many patients who suffer from diabetes mellitus are experiencing difficulties in managing the disease condition due to several factors including increasing cost and uncomfortable side effects of orthodox therapy. In addition, the increasing prevalence of this condition in the third world means there are more diabetics per orthodox health personnel and also increase in visits to diabetic clinics.[3-6]

In the light of these realities, there is an urgent need for a search for herbal recipes that could be used in the treatment and management of this metabolic disorder of the body. *Vernonia amygdalina* (Del.) which is widely grown in tropical Africa and known for its 'bitter principles'[7] is used in traditional medicine to treat fevers, measles, tuberculosis, toothaches, parasitic infections, asthma, diarrhoea and in managing diabetes mellitus.[7-10] Also, the extracts of the plant have been evaluated for laxative, arbortificient, anti-helmintic, antithrombotic and anticoagulant properties.[7-14]

The plant possesses nutritional values especially in the South-Eastern parts of Nigeria where its consumption is widespread.[8,10-11] Consequently, the present study was undertaken to investigate into the hypoglycaemic potentials of the leaf, stem and root extracts of *V. amygdalina* in rats.

MATERIALS AND METHODS

Plant Collection and Identification:

Fresh leaves, stem and roots of *V. amygdalina* were collected within the precinct of the botanical gardens of the Faculty of Pharmacy, University of Uyo, Nigeria around July, 2010. Voucher specimens of the plant (Nos H75-H77) were deposited in the herbarium of the Faculty of Pharmacy, University of Uyo, Nigeria.

Extraction

The fresh leaves of *V. amygdalina* were macerated with a wooden mortal and pestle after washing with water. 200g of the macerated leaves was squeezed and the resultant mixture filtered with a filter paper (Whatman International, England). The filtrate obtained was subsequently concentrated *in vacuo* on a rotary evaporator (Buchi CH-920, Laboratorium Technic, Flawk/SG, Switzerland) and the obtained dried powder stored in a silica-gel desiccator prior to further tests. Another 200g of the leaf extract was extracted with cold 96 % aqueous methanol at room temperature (27± 2 ^{0}C) for 72h, likewise concentrated and stored. The same procedures were repeated for the stem and roots.

Preparation of rats

Permission was sought from the College of Health Sciences' Animals Ethics Committee, University of Uyo, Uyo, Nigeria and approval was granted on the 24th, July, 2010 as contained in the reference document (UU/CHS/DP/12). The animals were then subsequently used in the

hypoglycaemic studies. Albino rats of both sexes obtained from the University of Uyo, Animal House weighing on the average 110.12 ± 10.67g were made diabetic by intraperitoneal injection of alloxan monohydrate (150mg/kg). The animals were quarantined for 7days to stabilize the blood glucose level. The rats were maintained under standard laboratory conditions and had free access to feed (Pfizer Feeds, Nigeria) and water *ad libtum*.

Administration of extracts

Normoglycaemic rats

The animals were arranged into seven groups of five rats each. The rats were put through a 12 h overnight fast and the groups were subsequently treated as follows:

Group I (control) - received 1ml of saline water orally.

Group II - received 300mg/kg of squeezed leaf extract orally.

Group III - received 300mg/kg of squeezed stem extract orally.

Group IV - received 300mg/kg of squeezed root extract orally.

Group V - received 300mg/kg of methanolic leaf extract orally.

Group VI - received 300mg/kg of methanolic stem extract orally.

Group VII - received 300mg/kg of methanolic root extract orally.

Diabetic rats

The animals were rested for 12 days and made diabetic by an inter-peritoneal administration of 150mg/kg alloxan monohydrate. After 5 days, the diabetic rats (glucose level >350mg/dL or 5.0 Mmol/L) were regrouped into four groups of 5 rats each. 15 of the animals had died on the 1st and 2nd days after the alloxan injection administration. The four groups were subsequently treated as highlighted below:

Group A (control) - received 1 ml of saline water orally.

Group B - received 300 mg kg^{-1} of methanolic leaf extract orally.

Group C - received 300 mg kg^{-1} of methanolic stem extract orally.

Group D - received 300mg kg^{-1} of methanolic root extract orally.

Estimation of blood glucose level

Blood was collected from the tail vein of the rats and analysed for glucose using the One Touch Glucometer (Ames Gx Model, Germany). In both the normal and diabetic rats (i.e., *a* and *b* above), blood glucose was determined at 0, 1, 2 and 4 hours.

Statistical analysis

The data were expressed as mean ± S.D. The significance of the data was determined using student's *t* – test and were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

The percentage changes (%) in blood glucose level are as displayed in Tables 2, 4 and 6. These values were calculated as follows:

$$\% \text{ Change} = 100\, G_T / G_O$$

where

G_T = Blood glucose level at time (t) = 1, 2 and 4 h;

G_O = Blood glucose level at time (t) = 0.

Normoglycaemic rats

The squeezed extracts of leaves, stem and roots of *V. amygdalina* were tested in normoglycaemic rats at 300mg/kg. The data obtained using the student's t test showed no statistically significant difference in the normoglycaemic rats when compared to the control at t = 1, 2 and 4 h as can be seen in Table 2. This might be due to the preparation technique adopted which could have hindered the amount of plant materials filtered into these squeezed extract mixtures. However, the methanolic extracts of leaves and roots exhibited significantly ($p<0.05$) and approximately similar hypoglycaemic activities in normoglycaemic rats especially at t = 2 and 4 h which are remarkable. These observations are as displayed in Table 4,

Diabetic rats

The methanolic extracts of leaves, stem and roots were equally tested in alloxan-induced rats at 300mg/kg. It could be seen that the hypoglycaemic activities of the leaf and root extracts were highly pronounced (80% reduction in blood glucose level) but was evidently poor in the stem extract (20% reduction in blood glucose level). These observations are as presented in Table 6. The methanolic leaf and root extracts demonstrated significantly ($p<0.05$) and approximately similar hypoglycaemic activities. These observations are not surprising because the extracts of *V. amygdalina* have been found to contain saponins, cardiac glycosides, tannins, flavonoids, terpenes, sugars, proteins, fats and vitamins C which have been implicated in previous studies to be hypoglycaemic.[15,16]

It is very probable that any of these chemical constituents or a combination of them could be responsible for the hypoglycaemic activity demonstrated by the plant. Further studies might have to be done to isolate and identify these hypoglycaemic principles and mechanism of action investigated

Table 1. Blood glucose level (mmol L^{-1}) in normoglycaemic rats using squeezed extracts.

Group	Extract	Dose	0 h	1 h	2 h	4 h
I	Control	1 ml saline water	2.5	2.4	2.4	2.5
II	Leaf	300 mg kg^{-1}	2.8	2.8	2.6	2.5
III	Stem	300 mg kg^{-1}	3.1	3.1	2.9	2.8
IV	Root	300 mg kg^{-1}	2.6	2.5	2.4	2.2

Mean ± S. D. $n = 5$

Table 2. Percentage change in blood glucose level ((mmol L^{-1}) in normoglycaemic rats using squeezed extracts.

Group	Extract	Dose	%	1 h	2 h	4 h
I	Control	1 ml saline water	100	96.3	98.2	99.3
II	Leaf	300 mg kg^{-1}	100	100	92.9	89.3
III	Stem	300 mg kg^{-1}	100	100	93.6	90.3
IV	Root	300 mg kg^{-1}	100	96.1	92.3	84.6

Mean ± S. D. $n = 5$

Table 3. Blood glucose level (mmol L^{-1}) in normoglycaemic rats using methanolic extracts.

Group	Extract	Dose	0 h	1 h	2 h	4 h
I	Control	1 ml saline water	2.5	2.4	2.4	2.5
V	Leaf	300mg/kg	2.8	2.7	2.5	2.1
VI	Stem	300mg/kg	2.8	2.7	2.6	2.7
VII	Root	300mg/kg	3.3	2.9	2.9	2.6

Mean ± S. D. $n = 5$

Table 4. Percentage Change in Blood Glucose Level ((Mmol/L) in Normoglycaemic Rats using Methanolic Extracts.

Group	Extract	Dose	%	1 h	2 h	4 h
I	Control	1 ml saline water	100	96. 2	98.2	99.3
V	Leaf	300mg/kg	100	96.4	89.3	75.1
VI	Stem	300mg/kg	100	96.4	92.9	96.4
VII	Root	300mg/kg	100	87.9	87.9	78.8

Mean ± S. D. $n = 5$, $p<0.05$

Table 5. Blood glucose level (mmol L^{-1}) in alloxan-induced rats using methanolic extracts.

Group	Extract	Dose	0 h	1 h	2 h	4 h
A	Control	1 ml saline water	11.6	10.5	10.8	10.5
B	Leaf	300mg/kg	11.6	8.8	7.4	3.5
C	Stem	300mg/kg	12.5	11.1	10.8	8.9
D	Root	300mg/kg	11.8	9.8	6.5	3.8

Mean ± S. D. $n = 5$

Table 6. Percentage change in blood glucose level ((mmol L^{-1}) in alloxan-induced rats using methanolic extracts.

Group	Extract	Dose	%	1 h	2 h	4 h
A	Control	1 ml saline water	100	90.8	93.1	90.3
B	Leaf	300mg/kg	100	76.3	63.7	30.1
C	Stem	300mg/kg	100	88.3	86.0	71.2
D	Root	300mg/kg	100	83.1	55.3	32.0

Mean ± S. D. $n = 5$, $p<0.05$

Furthermore, the results from this study as displayed in Tables 4 and 6 have shown that only the leaf and root extracts have demonstrated remarkable hypoglycaemic activities. This observation clearly negates the claims in [10] that the stem in addition to the leaves and roots *V. amygdalina* are employed in the treatment and management of diabetes mellitus especially in South-Eastern parts of Nigeria. Alloxan destroys the beta cells of the pancreas leading to insulin defiency.[4-5] Therefore, it is conceivable that the hypoglycaemic activities shown by the leaf and root extracts were not related to insulin secretion by the pancreatic cells but rather by other mechanisms of action. However, close monitoring of blood glucose concentration in humans is required in the use of the leaf and root extracts of *V. amygdalina* in the herbal therapy of diabetes mellitus to avoid hypoglycaemic shock.

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NUMERICAL CALCULATIONS OF IMPURITY SCATTERING MOBILITY IN SEMICONDUCTORS

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Keywords: semiconductor, current carriers mobility, impurity ions, numerical calculations

Novel semiconductor-base nanotechnology is gradually moving into new applications in the world economy. Semiconductor application requires increasing of investigations in the direction of their properties. The primary criterion of semiconductor suitability for use in semiconductor devices is its electrical properties, particularly current carriers mobility. Therefore, the problem connected with the explanation of the experimental results of current carriers mobility on the base of theoretical formulas is very urgent. In the present paper current carriers mobility due to ionized impurity scattering is discussed and calculated using numerical methods. Calculations have been done for different temperatures and different range of current carriers concentration in InAs.

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Introduction

An integral part of the new technologies, among them nanotechnology, are semiconductor devices. They have no alternative because of the economy in electric power consumption, compactness of the equipment on accounts of the extraordinary density of element packing in circuits, longevity, full automation, simplicity in operation, duration of activity without maintenance, high reliability, and so on. As dimensions of devices shrink to the nanometer range, the range of their applications broadens. Wherein the principle of functioning of all semiconductor devices is determined by the electrical properties of the active part of devices-semiconductor material, in particular, by the charge carriers mobility. Mobility determines the process of directed motion of charged particles in a semiconductor under the action of an electric field and gives enormous information about investigated materials. Therefore, a great practical and theoretical interest is the study of those processes that occurs when an electric field is applied to charge carriers. In this case, the charge carriers are in nonequilibrium conditions and transport phenomena arise that are related to the directional displacement of the charge carriers. There are several theoretical approaches to the study of transport phenomena.[1,2] The most common among them is the method of the Boltzmann kinetic equation, by means of which it is possible to calculate the mobility of charge carriers. An essential feature of nonequilibrium processes is that they depend substantially on the mechanism of interaction of the current carriers in the solid-state system, namely, their scattering by lattices such as atomic vibrations, impurity ions, etc.

The calculation of mobility components is needed not only in the study of the theory of semiconductors but at the explanation of the experimental results of investigated transport phenomena in semiconductors. However appropriate components of mobility are expressed by very complicated formulas and their treatment requires a lot of time. In the age of computer technology, it is reasonable and necessary to use modern software tools, a universal package for analyzing and managing databases, developing custom applications, containing a wide range of analysis procedures for use in scientific research in order to interpret the experimental results obtained with high accuracy. Therefore, the goal of our paper is to calculate impurity scattering mobility numerically.

Theoretical introduction to ionized impurity scattering

Of all possible scattering mechanisms of current carriers in semiconductors, scattering on ions of impurities practically always takes place in all semiconductors. The only exception is the very low temperatures near the temperature of the liquid helium. The electrical properties of semiconductors are determined by the presence of donor or acceptor impurities introduced into it. The reason is that impurity conductivity, as a rule, far exceeds the own conductivity of the semiconductor. The intrinsic conductivity of semiconductors is usually small, since the number of free electrons, for example, at room temperature is of the order of 10^{13}–10^{14} cm^{-3}. At the same time, the number of atoms in $1cm^3$ is ~10^{23} atoms.

Impurity centers can be atoms or ions of chemical elements embedded in the lattice of a semiconductor, excess atoms or ions implanted in the interstices of the lattice and various other defects and distortions in the crystal lattice (empty knots, cracks, shifts arising when deformations of crystals, etc.). The technique of semiconductor devices requires semiconductors both of maximum purity and doped. As the degree of doping increases, the density of the current carriers increases. At low impurity concentrations, due to the considerable distance between the impurity atoms, there is no interaction between them. Impurities form local states in the forbidden band. Because of the small number of charge carriers in the allowed band, they obey to the Boltzmann statistics. When the degree of doping is increased, the distance between impurity atoms is reduced, that leads to interaction between them, overlapping

of wave functions of charge carriers. The law of the charge carrier distribution with respect to energies in the impurity band and the allowed zones obey to the statistics somewhat between Boltzmann and Fermi–Dirac.[1–3] A substantial increase in the concentration of impurities leads to the confluence of the impurity band with the allowed band, and an allowed zone is formed.

In this case, a large concentration of charge carriers obeys to the Fermi-Dirac statistics and the gas of such particles is called degenerate. Thus, the properties of the electron gas significantly differ in undoped and doped semiconductors. The reduced Fermi level ξ defines the degeneracy criteξrion. A clear division into degenerate and non-degenerate charge carrier gases is conditional and depends on the temperature. As the temperature increases, when the intrinsic conductivity appears, the particles distribution in the electron gas will approach to Boltzmann statistics and, conversely, as the temperature decreases, the particles distribution will increasingly differ from Boltzmann's. It is interesting that in semiconductors the charge carriers gas becomes degenerate at low temperatures. It is accepted to assume with error 8 %, that $\xi = -2$ is the degeneracy boundary for charge carrier gas between degeneracy and no degeneracy state. At $\xi < -2$ the charge carrier gas is nondegenerate, at $\xi < -2$-degenerate. Different physical phenomena are differently sensitive to the form of the charge carrier distribution, and hence to the boundaries of degeneracy and will be ascribed differently by Fermi levels.

There exist many essential classical research works of Conwel and Weisskopf, Brooks–Herring (taking into account a screening effect), which considered the process of electron scattering by impurity centers in semiconductors.[4,5] However, these models are valid for charge carriers gas of noninteracting particles, which obey to the classical Maxwell-Boltzmann statistics and the charge carriers gas is non-degenerate. The impurity scattering in the case of non-degenerate charge carriers gas has been discussed by Mott.[6] However, often, impurity scattering has to be considered when it is not known when charge carriers gas is either degenerate or non-degenerate. Fortunately, there exists a Mansfield model for charge carriers scattering by impurity centers in semiconductor, which is valid for any distribution either degenerate or non-degenerate gas of current carriers in energy.[7] That is why in given work Mansfield model has been programmed.

Methodology

The expression of ionized impurity scattering mobility is:[6]

$$\mu = \frac{32\varepsilon^2 m^* (kT)^3 F_2(\xi)}{n^2 e^3 h^3 f(x)} \quad (1)$$

where

$$f(x) = \ln(x+1) - \frac{x}{x+1} \quad (2)$$

$$x = \frac{\eta (kT)^{1/2} \varepsilon_0 h}{e^2 (2m^*)^{1/2} F'_{1/2}(\overline{\eta})} \quad (3)$$

$$F'_{1/2}(\xi) = \frac{dF_{1/2}(\xi)}{d\xi} \quad (4)$$

In order to find the mobility values at different temperatures and for different concentrations of the current carriers, it is necessary first to calculate the reduced Fermi levels determined from the formula:

$$n = \frac{4\pi (2m^* kT)^{3/2} F_{1/2}(\xi)}{h^3} \quad (5)$$

where n is the charge carriers concentration, and -$F_{1/2}(\xi)$ integral Fermi.

From (5) equitation we derive:

$$F_{1/2}(\xi) = \frac{nh^3}{4\pi (2m^* kT)^{3/2}} \quad (6)$$

When the current carriers concentration is known at a given temperature and given effective mass, for finding the parameter ξ, it is necessary to solve the equation (6). For this, we transform it into:

$$F_{1/2}(\xi) - \frac{nh^3}{4\pi (2m^* kT)^{3/2}} = 0 \quad (7)$$

or

$$f(\xi) = 0 \quad (8)$$

where

$$\frac{nh^3}{4\pi (2m^* kT)^{3/2}} \equiv c, \; F_{1/2}(\xi) - c = 0, \; f(\xi) \equiv F_{1/2}(\xi) - c.$$

A solution of equation (8) gives the value of ξ parameter. We used the bisectors numerical method for the solution of (8) equitation. All programs were written in Matlab.

To calculate mobility (1) it is necessary to calculate another unknown parameter $\overline{\eta}$ which is in (3) equitation and it needs to solve transcendental equation (9) to find the value of $\overline{\eta}$:

$$(\overline{\eta} - 3) e^{\overline{\eta} - \xi} = (\overline{\eta} + 3). \quad (9)$$

After calculation of ξ parameter we can transform (9) equitation and define $\overline{\eta}$ using ξ value:

$$\xi = \overline{\eta} - \ln\left(\frac{\overline{\eta} + 3}{\overline{\eta} - 3}\right) \quad (10)$$

$$\overline{\eta} - \ln\left(\frac{\overline{\eta} + 3}{\overline{\eta} - 3}\right) - \xi = 0 \quad (11)$$

$$f(\overline{\eta}) = \overline{\eta} - \ln\left(\frac{\overline{\eta} + 3}{\overline{\eta} - 3}\right) - \xi \quad (12)$$

The root of the equation $f(\overline{\eta}) = 0$ gives the value of $\overline{\eta}$ which can be solved using the bisectors method.

The alternative way of solution of transcendental equation (9) is a graphical solution. But this method is not sufficiently accurate especially when we are interested in values of $\overline{\eta}$ at

meaning of arbitrary temperature and current carriers concentration. When solving (12) equitation, it is necessary to take into account that the equation is not defined in the region (–3, +3). These points need to be eliminated at the calculation by this method.

Results

As an example, the electrons mobility of *n*-InAs has been considered using this software. InAs is one of the semiconductors currently widely used in modern electronics and nanotechnology in the form of high-speed transistors and integrated circuits, IR photodetectors, injection lasers, among them in nanostructure of nanowires, structures with quantum dots InAs, etc. The behavior of the mobility for *n*-InAs due to scattering processes on impurity ions and its relation to temperature and doping concentration has been revealed. For this reason, there has been calculated mobility for temperatures: 77, 150, and 300 K for different values of electrons concentration in the range of 10^{16}–10^{19} cm^{-3}. When the electrons concentration changes in this interval, current carriers distribution in energy changes from non-degenerate to degenerate gas state. Results of the calculation of variation of the current carrier mobility with the concentration of electrons at different temperatures for *n*-InAs are presented in Figures 1–3.

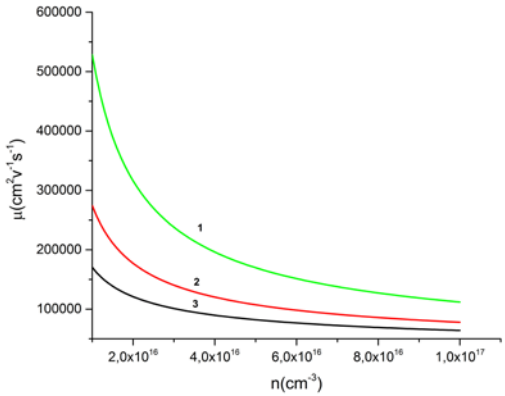

Figure 1. Calculated mobility due to scattering of electrons on impurity ions for electron concentration in the range of 10^{16}–10^{17} cm^{-3} at temperatures: 1 – 300 K, 2 – 150 K and 3 – 77 K.

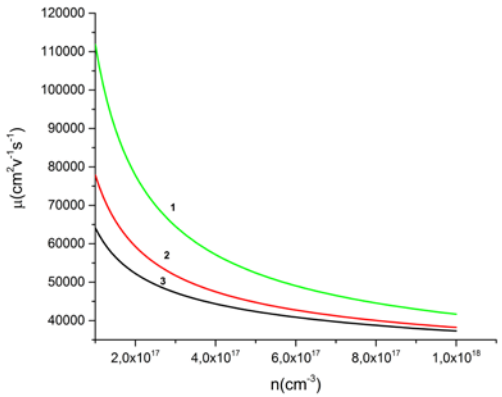

Figure 2. Calculated mobility due to scattering of electrons on impurity ions for electron concentration in the range of 10^{17}–10^{18} cm^{-3} at temperatures: 1 – 300 K, 2 – 150 K and 3 – 77 K.

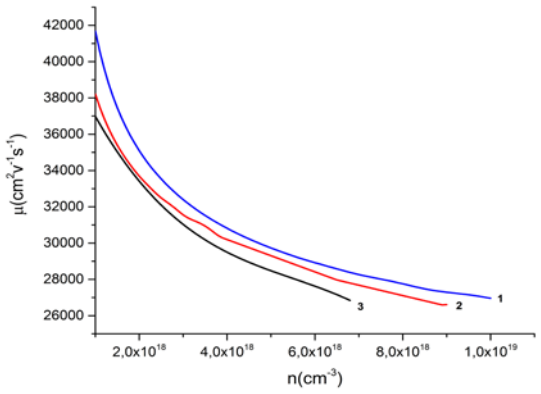

Figure 3. Calculated mobility due to scattering of electrons on impurity ions for electron concentration in the range of 10^{18}–10^{19} cm^{-3} at temperatures: 1 – 300 K, 2 – 150 K and 3 – 77 K.

It is clear that with rising temperature, mobility rises and with increasing of electrons concentration it falls. The results show that in experimental samples of InAs the impurities are all ionized in temperature range considered.[8]

The contribution of the scattering on the ionized impurity into the total scattering increases with increasing of impurity concentration. At n~$10^{17}$$cm^{-3}$ it is not still dominating. At the decrease of temperature below 300 K, the contribution of impurity ions in the scattering of carriers increases too. But with decreasing of T from 300 up to 77 K deionization of impurity levels takes place,

Comparison of experimental data of mobility in the InAs, containing an impurity in the range of 10^{16}–10^{19} cm^{-3} with the theoretical ones shows that, of all possible scattering mechanisms in InAs, the only combination of scattering on ionized impurities and optical phonons explains the experimental results in the temperature range.[8–10] The share of contribution of these scattering mechanisms into the total scattering is different at various temperatures and electrons concentration.

Conclusion

For interpreting the experimental results of current carriers mobility in semiconductors with high accuracy, there has been using modern software tools for numerical calculation of mobility due to impurity scattering. In given work model for mobility due to impurity scattering for the general case of any degree of degeneracy of the charge carriers has been programmed. The calculation has been made for the electrons mobility of *n*-InAs**.**

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STRESS-STRAIN CHARACTERISTICS OF BRICK MASONRY PREPARED WITH POND ASH IN CEMENT MORTAR UNDER UNIAXIAL COMPRESSIVE STRENGTH

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Keywords: Pond ash; cement mortar; compressive strength; Young's modulus.

The waste from coal-based thermal power plants in the form of pond ash is utilized in making of environment-friendly cement mortar with using sand and cement. The mechanical properties like compressive strength under uniaxial stress of brick masonry and cement mortars prepared with the incorporation of pond ash into the cement mortar at various mixing ratios have been determined. The mathematical relationship of compressive stress values and the composition of brick, mortar and masonry have been developed. The compressive stress, strain and Young's modulus values and their relationships have been determined. Using pond ash lead to an increase in the strength of the mortar and brick masonry prism prepared.

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Introduction

The usefulness of ash in building materials and civil engineering application has been known for a long time.[1,2] There are attempts to utilize pond ash in cement mortar in the brick masonry with replacement of cement and sand with various ratios as per IS 1905,[3] IS 2250,[4] Eurocode EC 6 and EN 1996-1-1.[5] Mathematical models were also developed to characterize the relationships between the compressive strength and modulus of elasticity of brick, mortar and masonry mortar contains alternative replacement materials.[6-14]

The present work includes the results about compression tests for masonry prisms prepared using reference mortar and pond ash modified mortar and the evaluation of relationships between the values of Young's modulus of elasticity (*E*) and the compressive strength for mortars prepared with various curing methods.

Experimentals

The locally available burnt clay brick, cement mortar with 1:4 proportion (contained one par cement and four parts sand) and brick masonry prism specimen were prepared. The samples of clay brick, mortar cubes and brick masonry prism were cured at 3, 7 and 28 days period. For the preparation of modified mortars, ordinary Portland cement (53 grade), river sand (locally available) and pond ash (sample collected from Thermal Power Plant, Bhusawal, Dist Jalgaon, Maharashtra) were used. Pond ash samples were dried after collection from disposal sites. The ratio between binder and filler used in the experiments was 1:4. The filler means river sand is first replaced with pond ash with a percentage level of replacement from no replacement to fifty percentage (abbreviation used as: $SR_{0\text{-}50}$). Replacement of cement at the same level named as $CR_{0\text{-}50}$. The mortar sample was prepared and cured at 7 and 28 d periods before testing. The compression test set up with a capacity of 400 kN was used to determine compressive strength and value of strain for burnt clay brick, cement mortar cube and brick masonry prism prepared using pond ash partially replaced with cement and sand. The samples were tested for their ultimate load carrying capacity. Stress and strain values were obtained and recorded. Test set up for brick masonry prism and cement mortar specimen can be seen in the Electronic Supplementary Information (ESI Fig.1.).

Result and discussion

Compressive strength values were measured and given in Table 1 and 2. The compressive strength values for pond ash modified products, the values decrease with increasing the replacement level for both $CR_{5\text{-}40}$ and $SR_{30\text{-}50}$ samples. The values of compressive strength for pond ash modified mortar exceeds the control in case of $SR_{5\text{-}35}$ samples. These values can be used to determine the elasticity modulus (*E*).

The K, α and β values as constants for model Eqn. 1 were determined with fitting the experimental values.

$$f_k = \mathrm{K}\, f_{\mathrm{b}}^{\mathbf{a}}\, f_{\mathrm{m}}^{\mathbf{b}} \qquad (1)$$

where,

f_{k} = characteristic strength of masonry prism (N mm^{-2})

f_{b} = Compressive strength of brick (N mm^{-2})

f_m= Compressive strength of mortar (N mm^{-2})

K=0.365

α=0.455 and

β=0.4

Table 1. Compressive strength values of prism, brick and mortar, along with a comparison of F_k (experimental and values obtained from Eqn. 2) for CR_{0-50}

Pond ash replacement	f_k experimental N mm^{-2}	Constant K	F_b N mm^{-2}	α	F_m N mm^{-2}	β	f_k from Eqn.2 N mm^{-2}	% Error
CR-0	2.61	0.365	4.19	0.455	24.53	0.4	2.52	3.36
CR-5	2.72	0.365	4.19	0.455	22.16	0.4	2.42	11.08
CR-10	2.28	0.365	4.19	0.455	19.81	0.4	2.31	-1.27
CR-15	2.42	0.365	4.19	0.455	15.89	0.4	2.12	12.47
CR-20	2.56	0.365	4.19	0.455	19.62	0.4	2.30	9.84
CR-25	2.22	0.365	4.19	0.455	19.36	0.4	2.29	-3.45
CR-30	1.88	0.365	4.19	0.455	19.11	0.4	2.28	-21.55
CR-35	1.79	0.365	4.19	0.455	16.99	0.4	2.17	-21.63
CR-40	1.70	0.365	4.19	0.455	14.87	0.4	2.06	-21.25
CR-45	1.47	0.365	4.19	0.455	13.14	0.4	1.96	-33.19
CR-50	1.42	0.365	4.19	0.455	10.56	0.4	1.80	-26.95

Table 2. Compressive strength values of prism, brick and mortar, along with a comparison of F_k (experimental and values obtained from Eqn. 2) for SR_{0-50}

Pond ash replacement	f_k experimental N mm^{-2}	Constant K	F_b N mm^{-2}	α	F_m N mm^{-2}	β	f_k from Eqn.2 N mm^{-2}	% Error
SR-0	**2.61**	**0.365**	**4.19**	**0.455**	**24.53**	**0.4**	**2.52**	**3.36**
SR-5	**3.15**	**0.365**	**4.19**	**0.455**	**26.91**	**0.4**	**2.61**	**16.96**
SR-10	**3.14**	**0.365**	**4.19**	**0.455**	**29.31**	**0.4**	**2.71**	**13.83**
SR-15	**3.19**	**0.365**	**4.19**	**0.455**	**31.69**	**0.4**	**2.79**	**12.51**
SR-20	**3.61**	**0.365**	**4.19**	**0.455**	**34.06**	**0.4**	**2.87**	**20.42**
SR-25	**3.63**	**0.365**	**4.19**	**0.455**	**39.14**	**0.4**	**3.04**	**16.45**
SR-30	**3.25**	**0.365**	**4.19**	**0.455**	**44.22**	**0.4**	**3.19**	**2.02**
SR-35	**3.06**	**0.365**	**4.19**	**0.455**	**38.21**	**0.4**	**3.01**	**1.71**
SR-40	**2.87**	**0.365**	**4.19**	**0.455**	**32.20**	**0.4**	**2.81**	**1.97**
SR-45	**2.63**	**0.365**	**4.19**	**0.455**	**29.44**	**0.4**	**2.71**	**-3.12**
SR-50	**2.41**	**0.365**	**4.19**	**0.455**	**25.70**	**0.4**	**2.57**	**-6.32**

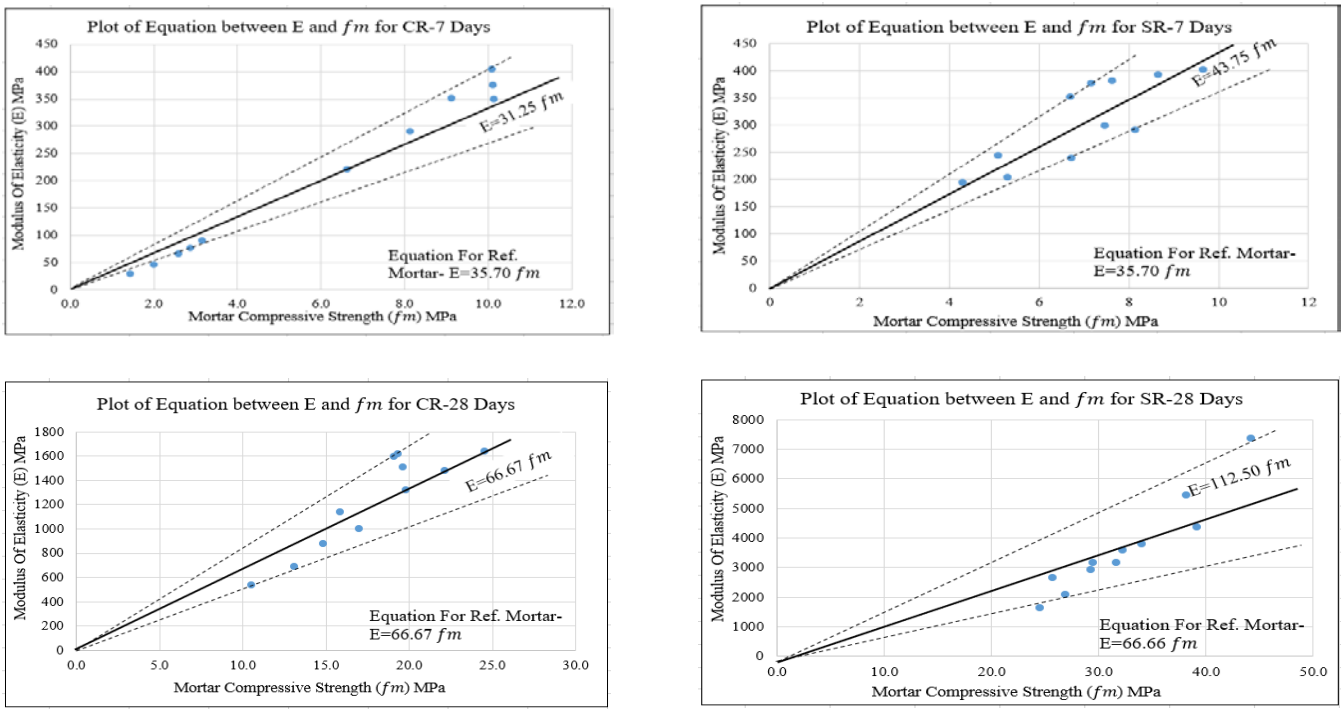

Figure 3. The plot of the equation between E and f_m for pond ash modified mortar for 7 and 28 days curing

The strength of brick prism is associated with the strengths of mortar and brick

The relations between elasticity modulus and strength for reference mortar/prism and pond ash modified mortar/prisms can be used for further analysis of masonry structures. The equations for 7 and 28 d curing period for reference and pond ash modified mortar/ prism samples are shown in Table 3-4.

Table 3. The equation for the relation between elasticity modulus (E) and Compressive strength (f_m) for cement mortar

Mortar mix	Reference mortar	Pond ash modified mortar
CR 7 Days	E=35.70 f_m	E=31.25 f_m
SR 7 Days	E=35.70 f_m	E=43.75 f_m
CR 28 Days	E=66.67 f_m	E=66.67 f_m
SR 28 Days	E=66.67 f_m	E=112.50 f_m

Table 4. The equation for the relation between elasticity modulus (E) and compressive strength (f_k) for brick masonry prisms

Mortar mix	Reference mortar	Pond ash modified mortar
CR 7 Days	E=83f_k	E=108 f_k
SR 7 Days	E=83 f_k	E=160 f_k
CR 28 Days	E=264f_k	E=266.67 f_k
SR 28 Days	E=264f_k	E=320 f_k

If the strength of mortar prism is known, the values for elasticity modulus can be calculated by using these equations available in Tables 3 and 4.

The compressive strength for pond ash modified mortar is higher for $SR_{5\text{-}40}$ than $CR_{5\text{-}50}$. The relation between elasticity modulus and compressive strength is obtained for 7 and 28 days are curing periods for brick masonry and mortar for reference and pond ash modified mortar. The new mathematical model obtained in accordance with Eurocode EC 6 and EN 1996-1-1 related to pond ash modified mortars for brick masonry prism.

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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SUBSTITUTED 2-PHENOXY-NICOTINALDEHYDES AS *α*-AMYLASE INHIBITORS

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Keywords: diabetes; *α*-amylase; *α*-glucosidase; 2-phenoxynicotinaldehydes.

Diabetes mellitus is a chronic endocrine disorder that affects the metabolism of carbohydrates, proteins, fat, electrolytes and water. *α*-Amylase and *α*-glucosidase are the crucial enzymes required for the digestion of the carbohydrate. These enzymes play a vital role in the breakdown of starch in the diet and its activity has been correlated to postprandial blood glucose levels, the control of which is essential for maintaining the quality of life for diabetic patients. We report the synthesis, characterization and biological evaluation of new substituted 2-phenoxynicotinaldehydes as *α*-amylase inhibitors. A new general method based on the aromatic nucleophilic substitution reactions of 2-chloronicotinaldehyde with differently substituted phenols in the presence of K_2CO_3 in dry dioxane was developed to furnish the corresponding substituted 2-phenoxynicontinaldehydes with 70-80% yields.

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Introduction

Diabetes Mellitus (DM) is an extended metabolic disease of several etiologies characterized by chronic hyperglycemia with a disorder of carbohydrate, fat and also protein metabolism. It includes a group of metabolic diseases characterized by hyperglycemia, in which blood sugar levels are elevated either from defects in insulin secretion, insulin action or both of them.[1] Therefore, it is necessary to decrease postprandial hyperglycemia to treat diabetes.[2] This can be achieved by the inhibition of carbohydrate-hydrolyzing enzymes like *α*-amylase and *α*-glucosidase.[3]

α-Amylase is responsible for the breakdown of long chain carbohydrates and *α*-glucosidase breaks down starch and disaccharides to glucose. They serve as the primary digestive enzymes and support in intestinal absorption. Both these enzymes are the potential targets in the development of lead compounds for the treatment of diabetes.[4]

Many natural products from plants have been used for the treatment of diabetes.[5-8] Various drugs are available for the cure of Type 2 diabetes like acarbose, biguanides, sulphonylureas, thiozolidinediones, etc.[9,10] But they have also exhibited many undesired side effects like gastrointestinal side effects and thus signifying other effective substitutes.[11]

The pyridine substructure is one of the most predominant heterocycles found in natural products, pharmaceuticals, and functional materials.[12] In the recent past, novel derivatives of pyridine have been developed and found to have a large number of biological activities.[13-20] The pyridine structure is found in natural compounds like nicotinic acid (vitamin B_3) and pyridoxine (vitamin B_6). Over 100 medications on the market today include pyridine rings, such as Lunesta, commonly used to treat insomnia,

Singulair, widely used to treat asthma, Nexium, widely used to treat acid reflux, and Actos, widely used to treat Type II diabetes (Figure 1).[21]

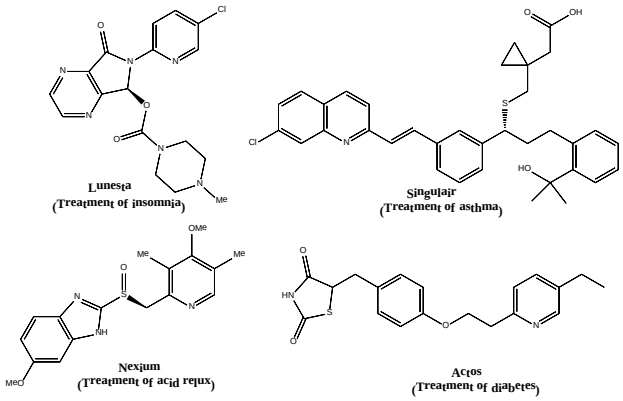


Figure 1. Some representative pyridine containing drugs

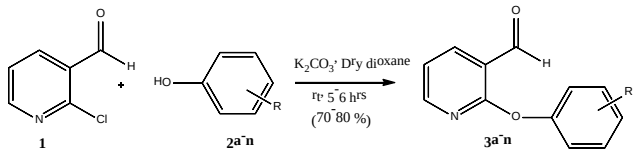
The pyridine moiety is also found in structurally simple drugs like isoniazid[22] and ethionamide[23] (both prodrugs for inhibitors of inter alia enoyl-acyl carrier protein reductase; tuberculosis), amrinone (phosphodiesterase 3 inhibitor; heart failure) and bupicomide (dopamine *β*-hydroxylase inhibitor; hypertension).

The high reactivity of pyridine allows for many possible chemical reactions.[24-25] In continuation with our efforts on the synthesis of bioactive heterocyclic compounds,[26-32] the present study was carried out to investigate the inhibitory potentials of substituted 2-phenoxynicotinaldehydes.

Results and discussion

We have synthesized a series of substituted 2-phenoxynicotinaldehydes by developing new reaction conditions. In the literature, various methods are reported for the aromatic nucleophilic substitution of 2-chloronicotinaldehydes by substituted phenols.[33-39] All these reported methods have some limitations. It requires high temperature and longer reaction time. Hence, there was a need to develop better reaction conditions for the synthesis of substituted 2-phenoxynicotinaldehydes from 2-chloronicotinaldehydes. In accordance with our aim, we performed the reaction of 2-chloronicotinaldehyde (**1**, 10 mmol) with phenol (**2l,** 10 mmol) in the presence of anhydrous K_2CO_3 (15 mmol) in dry dioxane at room temperature and exclusively obtained the corresponding 2-phenoxynicotinaldehyde (**3l**) with 75 % yield. In the same conversion, use of 10 mmol (1 equiv.) of K_2CO_3 also furnished the 2-phenoxynicotinaldehyde (**3l**) but less than 60 % yield, revealing that 15 mmol (1.5 equiv.) of K_2CO_3 is necessary for quantitative conversion of 2-chl**o**ronicotinaldehydes to corresponding substituted 2-phenoxynicotinaldehydes.

To establish the generality of this new set of reaction condition, we performed the aromatic nucleophilic substitution reactions of 2-chloro-nicotinaldehyde with differently substituted phenols in the presence of K_2CO_3 in dry dioxane to furnish the corresponding substituted 2-phenoxynicontinaldehydes with 70-80% yields (Scheme 1). ^1H and ^{13}C NMR spectral data confirmed the structures of all the synthesized substituted 2-phenoxynicontinaldehydes.



3a : R = 4-Cl; **3b** : R = 2, 4-Cl, Cl; **3c** : R = 2, 5-Cl, Cl; **3d** : R = 4-CH_3; **3e** : R = 2-Br; **3f** : R = 2-CH_3; **3g** : R = 3, 4-Cl, Cl; **3h** : R = 3-CH_3; **3i** : R = 2-CF_3; **3j** : R = 3, 5-Cl, Cl; **3k** : R = 2-CF3; **3l** : R = H; **3m** : R = 2-Cl; **3n** : R = 3-Cl

Scheme 1. Synthesis of substituted 2-phenoxy nicotinaldehydes

Biological activity

The enzyme inhibition activity was studied by agar diffusion method with some modifications.[40] For evaluating the enzyme inhibitory activity, commercially available *α*-amylase sample (from Hi media laboratory) was used. The synthesized compounds were dissolved in DMSO at 25 mg per ml concentration. A paper disc of 6 mm diameter from Hi media was impregnated with 10 µL of 1 % *α*-amylase solution. Subsequently, 10 µL of test compound solution was also impregnated to the enzyme discs. Control discs were prepared by adding 10 µL of DMSO only. Control and test discs were placed on 1 % starch containing Agar gel plates (pH 6.5). These plates were incubated at 37 ^{0}C for 24 h. After 24 h the plates were developed by Gram's iodine solution to observe the zone of clearance. Each zone was measured in millimetre (Table 1).

Table 1. Disc and medium preparation

Parameter	Magnitude
Concentration of enzyme	10 mg in 1 mL
Concentration of test compound	25 mg in 1 mL
Concentration of starch (substrate)	10 mg in 1 mL
Volume of the substrate in each plate	8 mL
Amount of substrate in each plate	80 mg
Diameter of zone (mm) for blank without enzyme with DMSO	Nil

The zone of control was used to calculate the amount of starch hydrolyzed. The amount of starch hydrolyzed was calculated as shown in Table 2. The zone of clearance indicated the amount of starch hydrolyzed in milligrams. The amount of starch hydrolyzed by control was considered as 100 % activity and accordingly, % change in activity was measured.

From Table 3, it is observed that all the tested compounds showed anti-*α*-amylase activity in the range from 25 % to 59 %. Among these compounds **2i** showed the least inhibition at 25.33 %, while compounds **2a**, **2e**, and **2g** showed higher inhibition of more than 59 %.

Table 2. Calculation for substrate consumed by control

Value of π	3.14
Thickness of medium, mm	1.01
Diameter of zone (mm) for control (with only amylase and DMSO)	22
Radius of zone (mm)	11
Volume of the reaction zone, mm^3, [πr^2*h (thickness of medium)]	383.7394
Amount of substrate consumed in control mg	383.7394/100

Table 3. Calculation of percent reduction in *α*-amylase activity

Entry	Diameter of the zone, mm	Consumed substrate, mg	Acti-vity, %	Activity fall, %
Control	22	3.83	100	0.00
2a	14	1.54	40.2	59.8
2b	15.5	1.89	49.34	50.66
2c	15.5	1.89	49.34	50.66
2d	16	2.01	52.48	47.52
2e	14	1.54	40.2	59.8
2f	16.5	2.14	55.87	44.13
2g	14	1.55	40.47	59.53
2h	15.5	1.9	49.6	50.4
2i	19	2.86	74.67	25.33
2j	15.5	1.89	49.34	50.66
2k	16.5	2.14	55.87	44.13
2l	17	2.27	59.26	40.74
2m	18.2	2.6	67.88	32.12
2n	17.6	2.43	63.44	36.56

Experimental

General procedure for the synthesis substituted 2-phenoxy-nicotinaldehydes

2-Chloronicotinaldehyde (10 mmol), substituted phenols (10 mmol) and potassium carbonate (15 mmol) in dry dioxane were stirred at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, the solvent was evaporated on a rotary evaporator. The reaction mixture was extracted by ethyl acetate. The crude product obtained was purified by recrystallization in ethanol to furnish the corresponding substituted 2-phenoxynicotinaldehydes with 70-80% yields.

2-(4-Chlorophenoxy)nicotinaldehyde (3a).

Yield: 74 %; M.p.: 80-82 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 7.15-7.19 (m, 3H), 7.41-7.45 (m, 2H), 8.27 (dd, *J* = 8 and 2 Hz, 1H), 8.36 (dd, *J* = 7 and 2 Hz, 1H), 10.56 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ ppm = 119.32, 119.52, 123.12, 129.80, 130.83, 138.34, 151.46, 152.99, 163.72, 188.52.

2-(2,4-Dichlorophenoxy)nicotinaldehyde (3b)

Yield: 70 %; M.P.: 122-124 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 7.17-7.20 (m, 1H), 7.26-7.28 (m, 1H), 7.34-7.37 (m, 1H), 7.51-7.52 (m, 1H), 8.28 (dd, *J* = 8 and 2 Hz, 1H), 8.31 (dd, *J* = 7 and 2 Hz, 1H), 10.60 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ ppm = 119.10, 119.58, 125.15, 128.18, 128.32, 130.41, 131.65, 138.41, 147.71, 152.85, 163.05, 188.33.

2-(2,5-Dichlorophenoxy)nicotinaldehyde (3c)

Yield: 70 %; M.P.: 88-90 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ ppm = 7.38 (dd, *J* = 8 and 5 Hz, 1H), 7.44 (dd, *J* = 8 and 2 Hz, 1H), 7.66-7.68 (m, 2H), 8.30 (dd, *J* = 8 and 2 Hz, 1H), 8.40 (dd, *J* = 5 and 2 Hz, 1H), 10.44 (s, 1H).

2-(*p*-Tolyloxy)nicotinaldehyde (3d)

Yield: 80 %; M.P.: 78-80 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 2.41 (s, 3H), 7.10-7.14 (m, 3H), 7.26-7.28 (m, 2H), 8.26 (dd, *J* = 8 and 2 Hz, 1H), 8.37 (dd, *J* = 5 and 2 Hz, 1H), 10.59 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ ppm = 20.94, 118.79, 119.45, 121.45, 130.31, 135.13, 138.04, 150.73, 153.19, 164.36, 188.94.

2-(2-Bromophenoxy)nicotinaldehyde (3e)

Yield: 76 %; M.P.: 83-85 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 7.15-7.22 (m, 2H), 7.23-7.34 (m, 1H), 7.42-7.46 (m, 1H), 7.68-7.70 (m, 1H), 8.28-8.34 (m, 2H), 10.65 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ ppm = 116.68, 119.23, 119.29, 124.38, 127.14, 128.67, 133.72, 138.21, 150.18, 152.95, 163.36, 188.68.

2-(*o*-Tolyloxy)nicotinaldehyde (3f)

Yield: 78 %; M.P.: Thick oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 2.23 (s, 3H), 7.10-7.20 (m, 2H), 7.22-7.34 (m, 3H), 8.27 (dd, *J* = 8 and 2 Hz, 1H), 8.34 (dd, *J* = 5 and 2 Hz, 1H), 10.64 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ ppm = 16.51, 118.74, 119.13, 122.13, 125.86, 127.20, 130.75, 131.46, 138.17, 151.44, 153.30, 163.98, 188.77.

2-(3,4-Dichlorophenoxy)nicotinaldehyde (3g)

Yield: 72 %; M.P.: 82-84 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 7.09-7.20 (m, 1H), 7.20-7.22 (m, 1H), 7.37 (d, *J* = 2 Hz, 1H),7.52 (d, *J* = 8Hz, 1H), 8.28 (dd, *J* = 8 and 2 Hz, 1H), 8.36 (dd, *J* = 5 & 2Hz, 1H), 10.53 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ ppm = 119.51, 119.72, 121.45, 124.04, 129.32, 130.97, 133.23, 138.52, 151.68, 152.92, 163.23, 188.18.

2-(*m*-Tolyloxy)nicotinaldehyde (3h).

Yield: 75 %; M.P.: 54-56 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 2.42 (s, 3H), 7.01-7.10 (m, 2H), 7.12-7.16 (m, 2H), 7.34-7.38 (m, 1H), 8.26 (dd, *J* = 8 and 2Hz, 1H), 8.37 (dd, *J* = 5 and 2 Hz, 1H), 10.58 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ ppm = 21.43, 118.59, 118.91, 119.57, 122.19, 126.33, 129.46, 138.06, 140.00, 153.07, 153.23, 164.23, 188.90.

2-(3-(Trifluoromethyl)phenoxy)nicotinaldehyde (3i)

Yield: 70 %; M.P.: 50-52 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 7.19-7.22 (m, 1H), 7.29-7.62 (m, 4H), 8.29 (dd, *J* = 8 and 2Hz, 1H), 8.37 (dd, *J* = 5 and 2 Hz, 1H), 10.57 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ ppm = 118.92, 119.00, 119.60, 119.63, 122.17, 122.24, 125.26, 130.24, 138.47, 152.94, 153.13, 163.36, 188.31.

2-(3,5-Dichlorophenoxy)nicotinaldehyde (3j)

Yield: 70 %; M.P.: 110-112 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 7.17-7.22 (m, 2H), 7.23-7.24 (m, 1H), 7.29-7.30 (m, 1H), 8.28 (dd, *J* = 8 and 2 Hz, 1H), 8.39 (dd, *J* = 5 and 2 Hz, 1H), 10.51 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ ppm = 119.62, 119.95, 120.83, 125.84, 135.50, 138.57, 152.96, 153.80, 162.98, 188.04.

2-(2-(Trifluoromethyl)phenoxy)nicotinaldehyde (3k)

Yield: 77 %; M.P.: 50-52 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 7.17-7.21 (m, 1H), 7.39-7.43 (m, 2H), 7.65-7.67 (m, 1H), 7.69-7.78 (m, 1H), 8.29 (dd, *J* = 8 and 2 Hz, 1H), 8.34 (dd, *J* = 5 and 2 Hz, 1H), 10.58 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ ppm = 119.57, 121.76, 124.47, 125.52, 127.18, 127.23, 127.28, 133.03, 138.24, 150.32, 152.77, 163.43, 188.47.

2-Phenoxynicotinaldehyde (3l)

Yield: 75 %; M.P.: 58-60 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 7.11-7.14 (m, 1H), 7.18-7.19 (m, 1H), 7.20-7.21 (m, 1H), 7.26-7.30 (m, 1H), 7.44-7.48 (m, 2H), 8.25 (dd, J = 8 and 2 Hz, 1H), 8.35 (dd, J = 5 and 2 Hz, 1H), 10.57 (s, 1H).

2-(2-Chlorophenoxy)nicotinaldehyde (3m)

Yield: 70 %; M.P.: 63-65 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 7.13-7.16 (m, 1H), 7.23-7.27 (m, 1H), 7.27-7.32 (m, 1H), 7.35-7.38 (m, 1H), 7.50-7.52 (m, 1H), 8.27 (dd, J = 8 and 2 Hz, 1H), 8.31 (dd, J = 5 and 2 Hz, 1H), 10.62 (s, 1H).

2-(3-Chlorophenoxy)nicotinaldehyde (3n)

Yield: 70 %; M.P.: 56-58 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 7.01-7.13 (m, 1H), 7.15-7.19 (m, 1H), 7.23-7.25 (m, 1H), 7.26-7.27 (m, 1H), 7.35-7.40 (m, 1H), 8.26 (dd, J = 8 and 2 Hz, 1H), 8.36 (dd, J = 5 and 2 Hz, 1H), 10.53 (s, 1H).

Conclusions

We have reported a new method for aromatic nucleophilic substitution of 2-chloronicotinaldehyde by substituted phenols to furnish the corresponding substituted 2-phenoxynicotinaldehydes. All the synthesized compounds showed anti-α-amylase activity. Among these compounds **2a**, **2e** and **2g** showed very good inhibition of more than 59%. These three compounds can be subjected to *in-vivo* studies.

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ELABORATION OF OPTIMAL MODE FOR HEAT TREATMENT OF SHALES FOR OBTAINING METAKAOLIN

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Production of metakaolin to increase the properties of cement and mortar has been studied from ordinary multicomponent mineral clays and shales as cheap raw materials. Alluvium clay shales formed as a result of mudflows were used as starting material and the optimal mode of its heat treatment has been elaborated to obtain the maximal amount of metakaolin. The pozzolanic properties of the heat-treated clay shales were monitored with making and testing various types of cement.

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INTRODUCTION

Portland cement concrete products and reinforced concrete structures are considered as the most widely used building materials of the modern construction industry. The main advantages of Portland cement based concrete the reliability and durability, the resistance towards aggressive environments, the high physical and mechanical properties and the possibility of regulating key characteristics. Despite many remarkable features and accessibility of raw material components, the global problem is that concrete belongs to energy- and material-consuming construction materials. Herewith, the most expensive and energy intensive component of the concrete is cement, more precisely, its basis - clinker. In order to improve the construction and technical characteristics of types of cement and give them specific properties such as sulfate resistance, water resistance, durability, etc., a variety of pozzolan admixtures have been added, which additives effectively reduce the consumption of the clinker part of the cement, reduce fuel consumption.[1,2]

A variety of materials containing silicon dioxide like geopolymers can be used as pozzolanic admixtures in cement production[3–6] and other processes in the production of fertilizers, detergents, special glasses, ion exchangers and catalysts.[7–10] In recent years, metakaolin as an active pozzolanic admixture to Portland cement has become very popular worldwide.[11,12] Metakaolin creates an opportunity to increase the density, water resistance and strength of cement (due to its high specific surface area – up to 13000 cm^2/g), and using it decreases the consumption of clinker. Metakaolin is obtained by heat treatment of kaolin clays, but the kaolin clay deposits availability is highly limited.

Therefore, studies have been carried out to obtain metakaolin from ordinary multicomponent mineral clays and shales.[13,14]

EXPERIMENTAL PART

The thermal studies have been performed on a MOM Q-1500D derivatograph (Hungary), with 10 ^{0}C min heating rate, in the air atmosphere, and with alumina standard.

The X-ray phase analyzes were carried out using a Dron 1.5 diffractometer (“Burevestnik”, St. Petersburg, Russia), with a Cu-anode and a graphite monochromator, intensity - 500 imp/sec, time constant - 5 s, U = 35 kV, I = 20 mA. λ = 1,54778 Å.

RESULTS AND DISCUSSIONS

Clay shales formed as a result of the accumulation of rocks collapsed due to mudflow stream to obtain metakaolin were removed from the bed and banks of the river Duruji. The phase analysis (XRD showed that the shales were the mixtures of hydromica, muscovite, biotite, pyrite, limonite, quartz, augite, sericite, calcite, plagioclase, orthoclase, chlorite. The chemical composition of shales is presented in Table 1.

The differential thermal analysis shows an endothermic effect between 100 and 150 °C corresponds to the removal of the physically or crystallization water. It is 4 % weight loss in the temperature range of 440–680 °C due to loss of constitutional water, while at 560–650 °C temperature an exothermic effect shows the burning out of organic inclusions and oxidizing of iron(II) content. In the temperature range of 680–730°C there is noted an endothermic effect, which is the result of the destruction of the crystalline lattice and proceeding of active amorphization.

Table 1. Chemical composition of shales, wt.%.

L.O.I.	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	FeO	P_2O_5	MnO	CaO	MgO	SO_3	Na_2O	K_2O
0.40	59.95	1.02	17.30	5.80	1.30	0.26	0.21	1.53	2.43	0.30	2.20	2.20

Based on the abovementioned results, the temperature range for the treatment of raw materials was selected to be between 600 and 800 °C. According to this, the temperature treatment of shales was performed at 600, 700 and 800 °C for 2 and 3 h.

X-ray phase analysis showed phase transformations of clay shales due to decomposition of components during the heat treatment (Figure 1). The diffractogram of natural shale (No.1) shows the presence of quartz (d=4.250, 3.340, 2.454, 2.285, 2.238, 2.128, 2.000, 1.985, and 1.817 Å), clay mineral chlorite (d=14.14, 7.08, 4.73, 3.54, 2.88, and 2.383 Å), mica (d=9.96, 4.96, 2.564, and 2.000 Å), and Ca–Na feldspar (d=4.03, 3.78, 3.67, 3.20, 2.954, 3.000, 2.930, and 2.395 Å). On diffractograms of samples heat-treated at 600 °C for 2 and 3 h (No.2 and 3, respectively), the amount of chlorite and mica decreases and an amorphous X-ray phase appears – the X-ray curve acquires a convex shape. On the diffractograms of samples heat-treated at 700 °C (2 and 3 h, No.4 and 5, respectively) the clay minerals completely disappear, the amount of mica is further reduced, and the amount of the amorphous phase is growing. The diffractograms of products formed at 800 °in 2 or 3 h (No. 6. and 7, respectively) are identical to diffractograms No.4 and 5, respectively. The decomposition of clay into amorphous pozzolanic oxides contain SiO_2, Al_2O_3, and Fe_2O_3, which are able to bind calcium hydroxide to form insoluble calcium hydrosilicates, starts at 600 °C and this process is completed by raising the temperature to 800 °C.

The reactivity of heat-treated clay shales towards lime is primarily due to the fact that at 600–800°C the main component of clay – inert kaolinite $Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$ – is dehydrated and turns into the active kaolinite anhydride – metakaolin ($Al_2O_3 \cdot 2SiO_2$), in its amorphized form as a result of the removal of water. Addition of metakaolin into cement compositions promotes the formation of new hydrated phases. The active silica reacts with lime to form calcium hydrosilicates, the active alumina forms a stable hydroaluminates and hydrogarnets. As a result of the reaction of Ca^{2+} and Al^{3+} ions with an amorphous silica content of metakaolin, new compounds are created, including the strong mineral stratlingite C_2ASH_8.[15,16]

X-ray phase analysis cannot be used to determine the amount of metakaolin due to its amorphous nature, but it is possible to determine the amount of active SiO_2 (Table 2) and the kinetics of its growth by the method of chemical analysis.[17]

According to Table 2, the maximum amount of active SiO_2 is formed in the temperature range of 700–800 °C. In this case, the exposure time is also essential; 2 h can be considered as optimal because with 3 h exposure, the sintering of the formed metakaolin occurs and it becomes less reactive. Thus, the optimal temperature treatment of clay shales was found to be 700-800°C with an exposure time of 2 h.

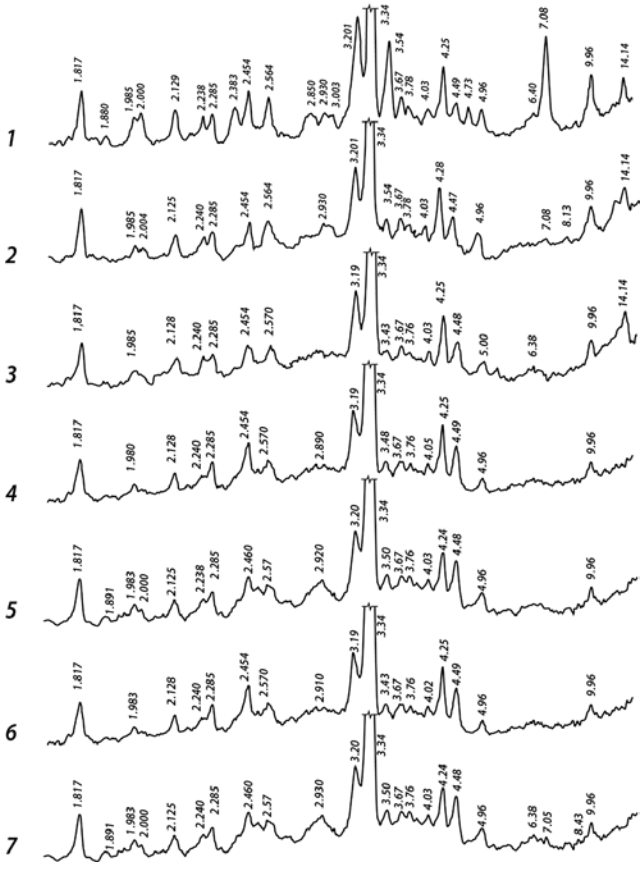

Figure 1. Diffraction patterns of shales: No.1 – natural (untreated shale), No.2 – heat-treated at 600 °C, 2 h exposure, No.3 – heat-treated at 600 °C, 3 h exposure, No.4 – heat-treated at 700 °C, 2 h exposure, No.5 – heat-treated at 700 °C, 3 h exposure, No.6 – heat-treated at 800 °C, 2 h exposure, and No.7 – heat-treated at 800 °C, 3 h exposure.

Table 2. Kinetics of growth of active SiO_2 with increasing temperature and exposure time.

No.	Treatment temperature, °C	Exposure, h	Amount of active SiO_2, wt.%
1	Untreated	–	10.21
2	600	2	16.88
3	600	3	20.64
4	700	2	26.77
5	700	3	21.00
6	800	2	26.93
7	800	3	19.56

To determine the pozzolanic properties of heat-treated clay shales, cement samples were made according to the ASTM C 311–05 standard. The test results are shown in Table 3.

Table 3. Physical-mechanical properties of cement samples.

Cement composition, wt. %	Compression strength, MPa		Strength activity index, %	
	7th day	28th day	7th day	28th day
Clinker – 95 Gypsum – 5	28.5	35.6	–	–
Clinker – 85 Shale* – 10 Gypsum –5	31.2	37.9	109.5	106.5
Clinker – 75 Shale* – 20 Gypsum-–5	24.1	31.5	84.6	88.5

* Shale was processed at 800 °C with an exposure time of 2 h.

According to ASTM C 618–05 the value of the "strength activity index" must obtain at least 75 % of the control mixture after 7 or 28 days.

CONCLUSION

The optimal mode of heat treatment of clay shales is heating at 800°C with an exposure time of 2 h. Shales processed in this way contain a certain amount of metakaolin and can be used as an active pozzolanic admixture to Portland cement.

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